# Radiation, biological diversity and host-parasite interactions in wild roses and insects

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**Inhalt**

**1. Einleitung ..... 1**

1.1 Radiationen und ihre Auswirkungen ..... 1

1.2 Geographische Diversitätsmuster ..... 2

1.3 Das Modelsystem der Wildrosen ..... 2

1.4 Gallenbildung durch *Diplolepis rosae* ..... 3

1.5 Die Hagebuttenfruchtfliegen *Rhagoletis alternata* und *Carpomya schineri* ..... 4

1.6 Fragestellungen und Ergebnisse ..... 5

1.7 Schlussfolgerung ..... 8

**2. Radiation, biological diversity and host-parasite interactions in wildroses, rust fungi and insects. ..... 9**

2.1 Introduction: Radiation, biodiversity and host-parasite interaction in the *Rosa*-system 10

2.2 Dog rosese are allopolylpoids: Genetic constitution of section *Caninae* ..... 11

2.3 Character inheritance in the heterogamous system of dog roses ..... 13

2.4 Glandular trichomes matter: Rust fungi on *Rosa* ..... 15

2.5 Evolution and diversity of plant-pathogen-insect foodwebs on dog roses ..... 15

2.6 How are the differences between the three closely related dog rose species translated into higher trophic levels? ..... 25

2.7 Conclusion ..... 27

**3. Cynipid gall-parasitoid interactions, comparing three dog rose species along a geographical gradient ..... 29**

**4. No host-associated differentiation in the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae) on three dog rose species ..... 43**

**5. Comparing geographical structures of one cynipid gall wasp with two specialised parasitoids in Europe ..... 56**

**6. No genetic differentiation in the rose-infesting fruit flies *Rhagoletis alternata* and *Carpomya schineri* (Diptera: Tephritidae) across central Europe ..... 77**

**7. Summary ..... 84**

**8. Referenzen ..... 88**

**9. Appendix ..... 104**

**Erklärung zu eigenen Beiträgen und Veröffentlichungen ..... 109**

**Erklärung ..... 111**

**Danksagung ..... 112**

**Lebenslauf ..... 114**

**Publikationsliste ..... 115**

# 1. Einleitung

## 1.1 Radiationen und ihre Auswirkungen

Aus einem komplizierten Geflecht gegenseitiger Abhängigkeit verschiedenster Organismen haben sich zum Teil hochspezialisierte Interaktionen entwickelt. Dazu zählen Symbiosen, Mutualismen, parasitische und prädatorische Lebensweisen auf mehreren trophischen Ebenen. Gegenseitige Abhängigkeit kann zu einer Koevolution zwischen den interagierenden Partnern und damit zu Differenzierungen innerhalb von Arten führen. So wird angenommen, dass die hohe Artenvielfalt (Biodiversität) der heutigen Blütenpflanzen (Angiospermen) und ihrer abhängigen Insektenfauna durch Interaktionen beider Partner entstanden ist (z.B. Ehrlich & Raven 1964).

Für die Entstehung hoher Biodiversität durch Artbildung (Speziation) sind evolutionäre Prozesse, wie räumliche oder zeitliche Trennungen, notwendig. Räumliche Trennungen können sowohl durch geographische Barrieren, als auch durch Wirtswechsel verursacht werden. In Europa sind viele Arten durch den Klimawandel der letzten Eiszeiten geprägt (Hewitt 1996, Taberlet et al. 1998, Hewitt 2000). Phänotypische Unterschiede zwischen Wirtspflanzen können z.B. durch unterschiedliche Fruchtungs- oder Blühzeitpunkte zu zeitlichen Trennungen von sich adaptierenden Arten führen (Bush 1969, Drès & Mallet 2002). Durch Anpassungen an Umweltbedingungen und das Ausnutzen neuer ökologischer Nischen kann es zu einer Aufteilung einer Art in mehrere höher spezialisierte Arten kommen, sogenannten Radiationen. Bekannte Beispiele für Radiationen sind die Darwinfinken auf Galapagos (Grant 1986), die Kleidervögel auf Hawaii (Wagner & Funk 1995) und die Buntbarsche (Cichliden) der ostafrikanischen Seen (Fryer & Iles 1972). Aber auch viele Pflanzenarten haben während der letzten Eiszeiten Radiationen durchlaufen; ein Beispiel in Europa sind die Wildrosen der Sektion *Caninae* (Wissemann 2005, Ritz et al. 2005b).

Besonders wichtig für Speziationsprozesse scheint vor allem die genetische Diversität zu sein. Durch ein Radiationsereignis entsteht aus einer Art in relativ kurzer Zeit eine Vielzahl genetischer und phänotypischer Variabilität. Über die Auswirkungen genetischer Variabilität auf Populationsstrukturen wurde in den vergangenen Jahren viel geforscht (z. B. Barratt et al. 1999, Barrowclough et al. 2005, Zenger et al. 2005). Aufgrund der genetischen Diversität einer Art wurden Überlebens- und Aussterbeszenarien prognostiziert, Ausbreitungswege rekonstruiert und Anpassungsprozesse postuliert. Darüber hinaus stellt sich die Frage, wie sich eine relativ junge, hohe Diversität, entstanden durch eine Radiation, auf abhängige

Arten wie Herbivore, Räuber oder Parasiten auswirkt. Beginnen sich die abhängigen Arten an die neue Formenvielfalt zu adaptieren, spezialisieren sie sich auf einzelne Arten und führt dies in der Folge ebenfalls zu einer Radiation und damit zu hoher Diversität der abhängigen Arten?

## 1.2 Geographische Diversitätsmuster

Auch phylogeographische Muster und räumliche Strukturen abhängiger Arten können durch ihre Wirte geprägt sein (z.B. Nieberding et al. 2004). Diversitätsmuster vieler Tier- und Pflanzenarten in Europa sind stark durch die Temperaturschwankungen des Pleistozäns geprägt (Hewitt 1996). Durch die Bedeckung Europas mit Gletschern waren viele Arten gezwungen sich in wärmere, eisfreie Gebiete, die Refugialgebiete, zurückzuziehen. Die mediterranen Regionen der iberischen und der Apennin-Halbinsel, sowie der Balkan bildeten solche Rückzugsgebiete (z.B. Hewitt 1996, Oshida et al. 2005). Nach dem Ende der Kaltzeiten breiteten sich viele Arten über Rückwanderungsrouten wieder nach Mitteleuropa aus (Taberlet et al. 1998). Aufgrund der heutigen Diversitätsmuster innerhalb der Arten kann man Rückschlüsse auf ihre Refugialgebiete und ihre Rückwanderwege ziehen.

Zu erwarten wäre, dass interagierende Arten gleiche Umweltbedingungen und eine gemeinsame Historie teilen, zudem sollten Refugialgebiete und Rückwanderwege zusätzlich von der jeweils anderen Art abhängen. Zu erwarten wären gleiche oder zumindest ähnliche geographische Muster. Für mehrere Wirt-Parasiten-Systeme wurden solche Strukturen schon öfters gezeigt (Funk et al. 2000, Nieberding et al. 2004, LaJeunesse et al. 2004). Nun stellt sich die Frage, ob das auch auf Wirte und ihre Parasitoiden zutrifft, die ähnlich wie Parasiten auf ihren Wirt spezialisiert sind.

## 1.3 Das Modelsystem der Wildrosen

Für beide Fragestellung eignet sich das System der Wildrosen, speziell der Sektion *Caninae* (Gattung: *Rosa*, Sektion *Caninae* (DC.) Ser.) besonders, da diese Rosen zeitgeschichtlich betrachtet erst vor kurzem eine Radiation durchlaufen haben und eine hohe Diversität aufweisen (Wissemann 2005, Ritz et al. 2005b). Die Sektion *Caninae* ist auf Hybridisierungs-Ereignisse während und nach den Eiszeiten (Pleistozän) zurückzuführen (Ritz et al. 2005b). Ein Hinweis auf eine Entstehung der Sektion im Zuge der pleistozänen Trennungs- und Einwanderungsereignisse ist ihr allopolyploider Chromosomensatz (Gustafsson & Hakansson, 1942). Sie verfügen über einen fünf-fachen Chromosomensatz, der in einem komplizierten Meiose-Verfahren (Reifeteilung) weitergegeben wird. Dabei stammen 4/5 des Genoms von mütterlicher und nur 1/5 von väterlicher Seite (Ritz & Wissemann 2003). Man unterscheidet fast 200 Rosenarten, die auch heute noch in der Lage sind zu hybridisieren.

Untersucht werden sollte, inwieweit sich diese Diversität auf rosenspezifische Insekten, die Hagebuttenfruchtfliegen (*Rhagoletis alternata* Fall. 1820 und *Carpomya schineri* Loew. 1856) und die Rosengallwespe (*Diplolepis rosae* L.) mit ihren spezifischen Parasitoiden auswirkt. Untersucht wurde zum einen eine Differenzierung zwischen verschiedenen Wirtspflanzen, zum anderen eine geographische Differenzierung. Um eine Anpassung an verschiedene Rosenarten zu überprüfen (Konzept der Wirtsrassen), wurden drei Rosenarten ausgewählt (*Rosa canina* L., *R. corymbifera* Borkh. und *R. rubiginosa* L.). Alle drei Arten sind in Europa weit verbreitet und kommen häufig in größerer Anzahl in denselben Gebieten vor (Timmermann & Müller 1994). Trotz ihrer nahen Verwandtschaft unterscheiden sie sich in mehreren, für beide Insektenarten relevanten, Merkmalen (z. B. Fruchtungszeitpunkt, Behaarung, sekundäre Inhaltsstoffe; Wissemann et al. 2006, Timmermann 1998).

## 1.4 Gallenbildung durch *Diplolepis rosae*

Für multitrophische Untersuchungen sind die Gallen der Rosengallwespe *D. rosae* sehr gut geeignet, da in ihnen Parasitoide und Hyperparasitoide mehrerer trophischer Stufen zu finden sind. Zudem sollten sich Präferenzen für verschiedene Rosenarten durch das parthenogenetische Fortpflanzungssystem, induziert durch das Bakterium *Wolbachia,* schneller abbilden.

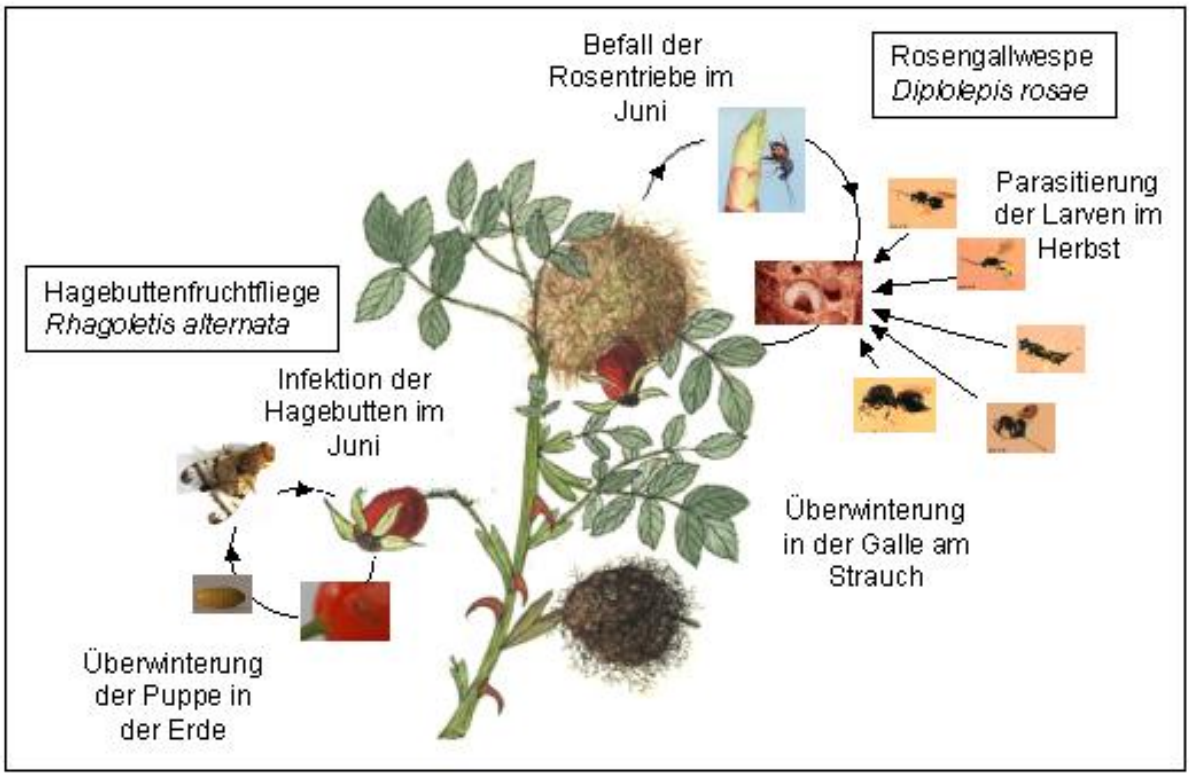


Abb.1.1. Lebenszyklen der rosespezifischen Hagebuttenfruchtfliege (*Rh. alternata*) und der Rosengallwepe (*D. rosae*). (Verschiedene Quellen © der Fotos u.a. Randolph, 2005; S.Rösner, A. Vaupel).

Weibchen der Rosengallwespe fliegen im Juni/Juli und legen ihre Eier in Sprosse der Wildrosen (Schröder 1967). Durch das Ablegen der Eier und das Schlüpfen der Larven wird die Bildung einer Pflanzengalle induziert (Abb.1.1). Das Gewebe der Rose beginnt zu wuchern und wächst zu einer ballförmigen Kugel mit filzigen Auswüchsen heran, die den Larven sowohl Nahrung durch spezielles Nährgewebe, als auch Schutz vor Fraßfeinden bietet (Randolph 2005). Im Herbst, wenn die Larven ausgewachsen sind, stirbt das Rosengewebe ab. Die Larven überdauern den Winter in der Galle. Im Frühling, ausgelöst durch wärmere Temperaturen, beginnen sich die Larven zu verpuppen und schlüpfen wenige Wochen später zwischen Mai und Juli. Trotz des Schutzes, den die Galle den Larven bietet, hat sich eine Reihe von anderen Insekten auf *Diplolepis*-Gallen spezialisiert. Neben dem Inquilin *Periclistus brandtii* Ratzeburg (Hym., Cynipidae) sind dies neun häufige Parasitoide (Askew 1960, Stille 1984). Während der Inquilin nur das Gallengewebe für seine eigenen Gallenkammern nutzt, ernähren sich die Parasitoiden-Larven von den Gallwespen-Larven. Die beiden häufigsten Parasitoide in den Gallen von *D. rosae* sind die Schlupfwespe *Orthoplema mediator* Thunb. (Hym. Ichneumonidae) und die Erzwespe *Glyphomerus stigma* Fabr. 1793 (Hym. Torymidae).

Induziert durch das Bakterium *Wolbachia* ist *D. rosae* in der Lage sich parthenogenetisch fortzupflanzen (Schilthuizen & Stouthamer 1998). Infizierte Weibchen können sich nicht mehr mit Männchen ihrer Art verpaaren. Sie legen unbefruchtete Eier, die sich mit Hilfe des Bakteriums zu diploiden Nachkommen entwickeln. Alle Nachkommen werden weiblich, was zu einer Abweichung vom 1:1 Verhältnis zwischen Männchen und Weibchen führt (McCallan 1940). Männchen werden immer seltener, während die Population fast ausschließlich aus Weibchen besteht. Es findet kaum noch genetischer Austausch statt, der bei anderen Tieren durch die Paarung und Rekombination sichergestellt wird.

## 1.5 Die Hagebuttenfruchtfliegen *Rhagoletis alternata* und *Carpomya schineri*

Die Hagebuttenfruchtfliegen *Rh. alternata* und *C. schineri* wurden als weitere rosenspezifische Arten ausgewählt. Erstere zählt zur selben Gattung wie die Apfelfruchtfliege (*Rhagoletis pomonella*; Walsh), dem Modellorganismus für Wirtsrassenbildung (Bush 1969). Beide Arten kommen in Südeuropa sympatrisch vor. Während *Rh. alternata* im gesamten paläarktischen Raum verbreitet ist (Kandybina 1977), ist *C. schineri* auf die Südpaläarktis beschränkt (White & Elson-Harris 1992). Beide Fruchtfliegen sind eng an ihre Wirtspflanze gebunden (Abb.1.1). Weibchen legen im Juni jeweils ein Ei in die noch grüne Hagebutte und markieren sie mit einem Pheromon (Bauer 1986). Die Larven ernähren sich ausschließlich vom Fruchtfleisch der Hagebutte und beschädigen die Samen nicht. Die Beziehung zwischen Fruchtfliegen und Wirtspflanze wird daher als „nicht-interaktiv“ bezeichnet. Dadurch hat die Larve nur einen geringen bis gar keinen Einfluss auf die Fitness der

Wirtspflanze (Bauer 1998). Die vollentwickelten Larven von *Rh. alternata* verlassen im Oktober, die von *C. schineri* bereits im August, die reife Hagebutte, um sich über den Winter in der Erde zu verpuppen (Hendel 1927; Bauer 1986). Trotz geringer Unterschiede zwischen Fliegen auf verschiedenen Rosenarten, konnten bisher für *Rh. alternata* auf der Basis von Allozymen keine Wirtsrassen nachgewiesen werden (Leclaire & Brandl 1994, Vaupel et al. 2007). Ebenfalls wurde mit Allozymen nur eine geringe Differenzierung von *Rh. alternata* zwischen Standorten innerhalb Deutschlands und der Schweiz nachgewiesen (Vaupel et al. 2007). Selbst die Alpen scheinen keine Barriere für die Rosenfruchtfliege zu bilden.

## 1.6 Fragestellungen und Ergebnisse

*1.6.1: Gibt es Unterschiede in der Befallsrate von* Diplolepis rosae *auf verschiedenen Rosenarten? Ist die Parasitierungsrate auf diesen Rosenarten unterschiedlich?* (Kapitel 3)

Um Wirtspräferenzen der Rosengallwespe *D. rosae* zu ermitteln wurden auf drei verschiedenen Rosenarten (*R. canina*, *R. corymbifera* und *R. rubiginosa*) an 17 Standorten in Deutschland die Anzahl der Gallen aufgenommen. Zusätzlich wurden Höhe, Blattdichte, Hagebuttendichte und Anzahl der Sprosse an je 5 Büschen ermittelt. An acht von diesen Standorten wurden alle Gallen abgesammelt, aufbewahrt und alle schlüpfenden Insekten (Gallwespen und Parasitoide) bestimmt und gezählt, insgesamt 6175 Individuen aus 388 Gallen.

Mit Hilfe eines Generalisierten Linearen Modells (GLM) wurde der Einfluss der Rosenart und des Standortes auf die Anzahl der Gallen pro Busch untersucht. Es zeigte sich, dass die Befallsrate mit Gallen stärker von der Form des Busches abhängt, als von der Rosenart. Büsche mit vielen austreibenden Ästen werden häufiger befallen, da sie den Gallwespen mehr Möglichkeiten zur Induktion von Gallen geben. Die Wuchsform eines Busches unterscheidet sich jedoch zwischen den Rosenarten. Im Mittel zeigt *R. rubiginosa* einen buschigeren und höheren Wuchs, wird somit im Mittel häufiger befallen als die anderen beiden Rosenarten. Die Größe der gebildeten Gallen ist jedoch unabhängig von der Rosenart.

In einer zweiten Untersuchung wurde der Einfluss der Rosenart auf die Parasitoide getestet. Ebenfalls mit einem GLM konnte gezeigt werden, dass die Parasitierungsrate nicht von der Rosenart, sondern vom Standort und vom Gallvolumen abhängt. Es besteht allerdings eine starke Interaktion zwischen der Rosenart und dem Standort. Die Zusammensetzung der Parasitoiden-Gemeinschaft ist vom Gallvolumen, aber auch vom Standort und der Rosenart abhängig.

Daraus kann geschlossen werden, dass sich Interaktionen multitrophischer Artengemeinschaften nicht zwischen Rosenarten unterscheiden. Wahrscheinlich unterscheiden sich die Wildrosen durch fortgesetzte Hybridisierungen nicht genug, um Präferenzen für einzelne Rosenarten auszubilden. Trotzdem zeigt die Wirtspflanze einen wichtigen Einfluss, der sich durch verschiedene Umweltbedingungen an verschiedenen Standorten auswirkt.

*1.6.2: Unterscheiden sich Gallwespen auf verschiedenen Rosenarten genetisch voneinander?* (Kapitel 4)

Im Herbst 2006 und 2007 wurden an fünf Standorten in Deutschland alle verfügbaren Gallen der drei Rosenarten (*R. canina*, *R. corymbifera*, *R. rubiginosa*) gesammelt. Die Gallen wurden in Plastikboxen mit Gazeverschluss (Luftaustausch) im Freien gelagert, um eine natürliche Entwicklung zu gewährleisten. Ab Mai des darauffolgenden Jahres schlüpften sowohl die adulten Gallwespen als auch die Parasitoide. Die geschlüpften Tiere wurden ausgezählt, sortiert und konserviert. Pro Standort wurden von jeder Rosenart maximal 15 (insgesamt 149) Individuen untersucht. Sie wurden auf ihren Befall mit dem parthenogenese-induzierenden Bakterium *Wolbachia* überprüft und mit fünf Primerkombinationen der populationsgenetischen Methode der AFLPs (Vos et al. 1995) analysiert. Die Befallsrate mit *Wolbachia* Bakterien betrug fast 100%. Dagegen zeigten die 106 polymorphen AFLP Marker weder genetische Differenzierungen zwischen Wirtspflanzen noch zwischen geographischen Standorten. Dies deutet auf eine gute Ausbreitungsfähigkeit u.a. durch Windverdriftung, sowie möglicherweise mehrere Infektions-Ereignisse mit erfolgreichen *Wolbachia*-Stämmen hin, die sich in der ganzen Population durchsetzen konnten. Die Wahl der Wirtspflanze scheint hierbei keine genetische Austauschbarriere zu sein, da es keine Spezialisierung von Gallwespen auf unterschiedliche Wirtspflanzen zu geben scheint. Ein Grund hierfür könnte die fortdauernde Hybridisierung zwischen verschiedenen Wildrosenarten sein.

*1.6.3: Sind die Diversitätsmuster von* Diplolepis rosae *und seinen häufigsten Parasitoiden* Orthopelma mediator *und* Glyphomerus stigma *europaweit ähnlich?* (Kapitel 5)

Alle drei Insektenarten sind in ihren Lebenszyklen eng miteinander verbunden und beide Parasitoide befallen ausschließlich Arten der Gattung *Diplolepis*, die wiederum alle auf Wildrosenarten angewiesen sind. Daher wurden ähnliche genetische Strukturen aufgrund ähnlicher Historien innerhalb Europas erwartet.

Gesammelte Gallen aus verschiedenen Ländern Europas wurden im Freien in Plastikboxen gelagert, so dass sich alle Larven entwickeln und schlüpfen konnten. Sodann wurden die Insekten aussortiert, bestimmt und in Ethanol konserviert. Von 79 *D. rosae* Individuen aus 17 europäischen Ländern, 56 *O. mediator* und 28 *G. stigma* Individuen wurde der Interne

Transkribierte Spacer 2 (ITS 2) aus dem Genom und die Cytochrom Oxidase I (COI) vom Mitochondrium sequenziert. Alle Individuen wurden auf ihren Befall mit *Wolbachia* Bakterien untersucht. Es zeigte sich, dass die Diversitätsmuster und genetischen Strukturen der drei Insektenarten innerhalb Europas sehr unterschiedlich sind. Die Gallwespe, *D. rosae*, zeigt eine geringe Variabilität und einen hohen Grad an Durchmischung, was auf eine gute Ausbreitungsfähigkeit hinweist. Sie ist fast komplett mit *Wolbachia* Bakterien befallen. In Südeuropa ist die genetische Diversität höher als in Zentraleuropa, was auf südliche Refugialgebiete hindeutet. Ganz anders ist das Muster des Parasitoiden *O. mediator*. Diese Insektenart zeigt eine klassische Ost-West Trennung zweier Linien in Europa mit einer Suturzone in Frankreich und Deutschland. Beide Linien sind deutlich differenziert, erklärbar durch zwei Refugialgebiete möglicherweise in Spanien und Osteuropa und ihre entsprechenden Rückwanderungsrouten. Der zweite Parasitoid, *G. stigma,* zeigt eine extrem hohe Variabilität, die keine Rückschlüsse auf räumliche Strukturen zuläßt und ist überhaupt nicht mit *Wolbachia* befallen. Die extremen Unterschiede in den Populationsstrukturen und deren Entkopplung ist unerwartet und nur durch ein Ausweichen auf andere Wirtsarten zu erklären.

*1.6.4: Wie groß ist die Diversität von Rosenfruchtfliegen europaweit?* (Kapitel 6)

Larven von *Rh. alternata* und *C. schineri* wurden in Deutschland, sowie den angrenzenden europäischen Ländern gesammelt. Befallene Hagebutten wurden in perforierten Plastikbeuteln bei ca. 15°C gelagert bis die Larven die Hagebutten verließen, um sich zu verpuppen. Bis zur Verwertung im Labor wurden die Puppen in 90% Ethanol gelagert. Es wurden *Rh. alternata* Larven von 12 Standorten und *C. schineri* Larven von fünf Standorten untersucht. Drei in der Regel sehr variable Gene (Cytochrom oxidase I und II, Cytochrom b), die sich für populationsgenetische Untersuchungen eignen (Simon et al. 1994, z.B. Lunt et al. 1996, Rokas et al. 2002, Simon et al. 2006), wurden sequenziert. Diese drei mitochondriellen Gene ergeben einen Sequenzabschnitt von 1720 Basenpaaren (bp). Es hat sich gezeigt, dass alle untersuchten *Rh. alternata* Individuen exakt die gleiche Basenpaarfrequenz aufweisen, also keinerlei Diversität gefunden werden konnte. Die Diversität von *C. schineri* war ebenfalls sehr gering, es konnten nur zwei Haplotypen gefunden werden. Ein Vergleich mit 61 anderen Insektenarten zeigt, dass dies eine außergewöhnlich geringe Variabilität ist. Dies ist ein sehr erstaunliches Ergebnis und nur zum Teil durch die gute Verbreitungsfähigkeit und hohen Populationsdichten der beiden Fruchtfliegen zu erklären.

## 1.7 Schlussfolgerung

Die grundlegende Fragestellung dieser Untersuchung war, in welchem Grad die hohe Variabilität der Wildrosen in den abhängigen Arten der nächst höheren trophischen Stufen wiederzufinden ist. Für keine der beide rosenspezifischen Insektenarten, weder für die Gallwespe *D. rosae*, noch für die Hagebuttenfruchtfliege *Rh. alternata* (Leclaire & Brandl 1994, Klinge 2005, Vaupel et al. 2007), konnten bisher Wirtspräferenzen oder gar Adaptationen gefunden werden. Ein ähnliches Ergebnis wurde von Ritz et al. (2005a) für Pilze der Gattung *Phragmidium* gefunden, sie zeigen ebenfalls keine Unterschiede auf verschiedenen Rosenarten. Auch die dritte trophische Ebene, die Parasitoide, wurden durch die Wirtspflanze kaum beeinflusst. Viel wichtiger scheint für die Artenzusammensetzung die Gallengröße zu sein. Zu erklären ist der Mangel an Adaptation durch die fortdauernde Hybridisierung der heutigen Rosenarten, die zu einem ständigen Genfluss zwischen den Arten führt und damit scharfe Grenzen zwischen den Arten verwischt. Hybride zwischen Wildrosen erben ihre phänotypischen Ausprägungen zum Großteil von der Mutterpflanze (Wissemann et al. 2006), jedoch sind alle Zwischenstufen durch fortlaufende Hybridisierung möglich. Das kann für die abhängigen Insekten einen Wechsel von einer Art auf eine andere Art ohne Schwierigkeiten ermöglichen.

Auch geographisch zeigt sich kein übereinstimmendes Bild in der genetischen Struktur sowohl zwischen den rosenspezifischen Insektenarten, als auch zwischen ihnen und ihren Parasitoiden. Damit sind keine Rückschlüsse auf eine Historie der Wirtspflanzen zu ziehen. Die Entkopplung und völlige Abweichung ist vermutlich auf unterschiedliche Ausweichmöglichkeiten in der Wirtswahl zurückzuführen. Einerseits können die rosenspezifischen Insektenarten zwischen verschiedenen Rosenarten, teilweise sogar Rosen anderer Sektionen, wechseln, andererseits sind auch die Parasitoide in der Lage andere Wirte der Gattung *Diplolepis* zu befallen, die wiederum ebenfalls andere Rosensektionen befallen können. Zudem haben alle untersuchten Insektenarten gute Ausbreitungsfähigkeiten aufgrund ihrer geringen Größe und damit guten Verdriftbarkeit durch Wind. Rosen, auch Hundsrosen wurden und werden immer noch in ihrer Ausbreitung stark vom Menschen beeinflusst. Sie werden u. a. in Gärten, Parkanlagen, als Hecken und entlang von Wegrändern und Eisenbahnlinien angepflanzt. Mit den Pflanzen können auch die Insekten leicht verbreitet werden. Das erklärt den hohen Grad an genetischem Austausch und die geringe Differenzierung zwischen den Standorten bei den meisten der untersuchten Insekten. Im System der Wildrosen und ihrer abhängigen Arten finden sich demnach weder wirtsspezifische, noch einheitliche geographische Differenzierungen in den höheren trophischen Ebenen wieder.

# 2. Radiation, biological diversity and host-parasite interactions in wildroses, rust fungi and insects.

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## ABSTRACT

One of the major tasks in evolutionary ecology is to explain how interspecific interactions influence the dynamics of evolutionary processes and enable radiation and genesis of biological diversity. The bewildering diversity of dog roses is generated by a heterogamous reproductive system. Genetic distance between rose taxa was analysed as base line for the explanation of subsequent radiation of the two host dependent parasite groups, rust fungi and insects. We investigated the interaction between each host-parasite system and between the parasite groups. We learned that the functional diploidy at the meiotic level is not reflected at the phenotypic level in dog roses. The phytophagous insect community shows only minor differences in composition on different rose species. These invertebrates seems not negatively affected by glandular trichomes, but for the rust fungi *Phragmidium* „glandular trichomes matter“, because they are negatively correlated with the infection. The abundance of two rose specialists the rose hip fly *Rhagoletis alternata* Fall. and the rose gall wasp *Diplolepis rosae* L. differed on rose species, but *Rh. alternata* showed neither any genetic differentiation on host species, nor geographical differentiation. As a basic result we detected that genetic diversity of dog roses is not translated into a hostspecific radiation of the parasites. We assume that intensive reticulate evolution of dog roses prevents cospeciation.

## 2.1 Introduction: Radiation, biodiversity and host-parasite interaction in the *Rosa*-system

A key topic in evolutionary ecology is to explain how interspecific interactions influence the dynamics of evolutionary processes and enable radiation and unfolding of biological diversity. In host-parasite interacting systems the most important questions is: How does the radiation and diversity of the hosts translate into the radiation and diversity of the parasites and what is the role of parasite interactions?

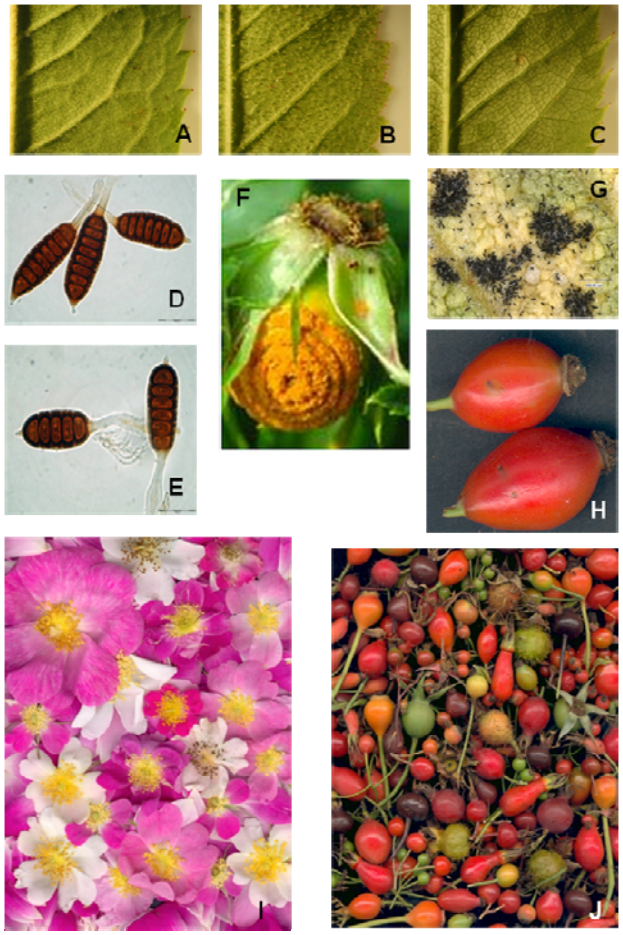


Fig. 2.1. **A**. lower surface of *R. corymbifera* (hairy), **B**. *R. rubiginosa* (glandular), **C**. *R. canina* (glabrous, eglandular). **D**. *Phragmidium mucronatum*, **E**. *Phragmidium tuberculatum*, **F**. rust infection on a hip of *R. canina*, **G**. Teliospores, **H**. *Rhagoletis alterna* infected hip of *R. canina*, **I**. Flower diversity in *Rosa*, **J**. Hip diversity

Such analyses are extremely rare due to the complexity of these systems (see e.g. Clay 1989, Pirozynski & Haksworth 1989). In this study we investigate the host-parasite net of dog roses (Rosaceae, Rosoideae, *Rosa* L., sect. *Caninae* (DC.) Ser.), rust fungi (*Phragmidium*), and insects. By analysing the genetic distance, variability and phylogenetic relationships between rose taxa, we determine a base line for the explanation of the subsequent radiation processes of two host dependent parasite groups, rust fungi and insects (Fig. 2.1). Understanding of the dog roses' radiation process enables us to unravel the levels of interaction (co-evolution, co-speciation, individualistic interaction) on which rust fungi and insects act. Dog roses are thought to have evolved during the Pliocene (5.3 -1.8 Mya) as a result of a single event, into which the peculiar mode of *Canina*-meiosis developed and then colonised Central Europe by a very fast and explosive radiation since the Pleistocene and Holocene (Zielinski 1985). Wissemann (2000a) and the study by Ritz et al. (2005b) showed that dog roses are permanent allopolyploids

arisen by multiple hybridisation events. The high genetic variability due to allopolyploidy and great homology between the different chromosome sets enabling interfertility between any dog rose species are the reasons for the morphological variation and the existence of numerous local forms.

Subsequent to the *Rosa* radiation, numerous pathogens interacted with their hosts. At present both, the analysed species of rusts and insects are found on any of the investigated dog rose species. Nevertheless, little is known about the genetic diversity of the two main (and commercially important) rust fungi on roses, *Phragmidium mucronatum* (Pers.) Schltdl. and *Phragmidium tuberculatum* J. Müller. We do not know anything about the radiation process of subspecific taxa (races, strains) and we do not know the level on which the fungi became host specific. The same is the situation in the numerous rose specific insect species. One group are the gall-forming insects, which are highly susceptible to plant resistance and are adapted to specific resources in most cases.

## 2.2 Dog rosese are allopolylpoids: Genetic constitution of the section *Caninae*

The genus *Rosa* consists of about 200 species following the classification by Wissemann and Ritz (2005). Based on morphological characters four subgenera are recognized: *Hulthemia* (Dumort.) Focke, *Platyrhodon* (Hurst) Rehder, *Hesperhodos* Cockerell, and *Rosa*. The subgenus *Rosa* comprises ten sections with more than 150 species of various ploidy-levels, distributed mainly in the temperate regions of the northern hemisphere. Studies involving excessive cloning of nuclear genes (ribosomal spacers and low copy genes) revealed that hybridisation played an important role in the evolution of polyploid rose taxa (Wissemann 2000a, Ritz et al. 2005b, Joly & Bruneau 2006, Joly et al. 2006). Within subgenus *Rosa*, members of the polyploid section *Caninae* are of particular interest, not only because of their unique meiotic behaviour but also for the readiness, with which members of the section can hybridize (Feuerhahn & Spethmann 1995, Wissemann & Hellwig 1997, Reichert 1998, Nybom et al. 2004, 2006, Werlemark & Nybom 2001, Werlemark et al. 1999). Early studies analysing nrITS-1 sequences revealed the existence of non-concerted evolution of the nrITS-region and thus confirmed the allopolyploid constitution of dog roses (Eigner & Wissemann 1999, Wissemann 1999, 2000b, 2002). Based on these studies we showed, that the tetra-, penta- or hexaploid dog roses arose by multiple hybridisations across the genus *Rosa* (Fig. 2.2, Ritz et al. 2005b).

Dog roses are cytologically characterised by a specific meiosis (Täckholm 1920, 1922, Klášterská 1969, 1971, Klášterská & Natarajan 1974, Roberts 1975). This meiosis leads to

heterogamous reproduction with (in the case of pentaploid roses, 2n=5x=35) haploid pollen grains (n=1x=7) and tetraploid egg cells (n=4x=28; reviewed in Wissemann & Ritz 2007).

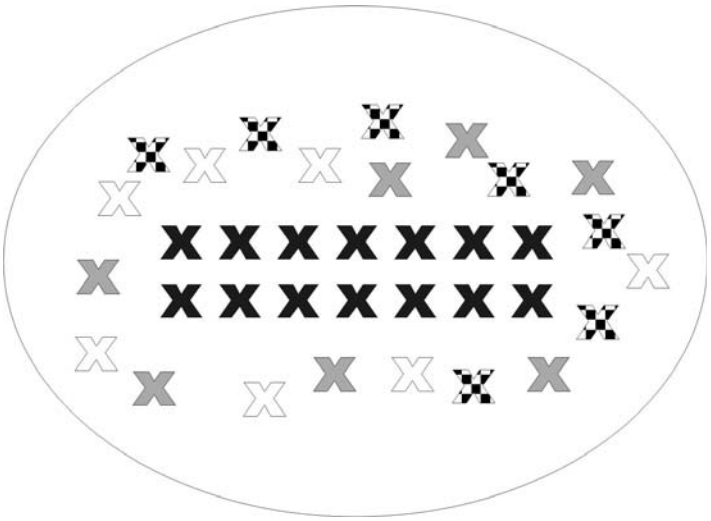

Fig. 2.2. Hypothetic genetic constitution of an allopolyploid pentaploid dog rose (2n=5x=35, x=7) with parental genomes from multiple hybridisation after Ritz et al. (2005a) and Kovarik et al. (2008). Black chromosomes: diploid bivalent forming Protocaninae genome, grey white and checked chromosomes: *woodsii*-, *rugosa*- and *gallica*- univalent forming genomes of other sections of the genus *Rosa*.

By microsatellite analysis (Ritz & Wissemann, submitted) we added further support to the first results of Nybom et al. (2004, 2006) that always the same chromosome sets pair during meiosis (bivalents) and the same three sets are unpaired (univalents). These findings support the hypothesis that dog roses are functional diploids: The bivalents are meiotically recombined and transmitted through the egg cell and the pollen grain, but the univalents are inherited apomictically by the egg cell only (Zielinski 1985, Nybom et al. 2004, Lim et al. 2005, Nybom et al. 2006). Thus, the canina meiosis combines two modes of reproduction: The interacting bivalents generate variability via sexual recombination and the apomictic univalents conserve information which allow for the tremendous variability and radiation possibilities. This unique meiosis of dog roses is presumably connected to a particular chromosome set characterized by the *canina*-nrITS type, which is not known to exist anymore in a diploid species (Ritz et al. 2005b). Present results show that the *canina*-nrITS type has at least two copies in each dog rose and is involved in bivalent formation (Kovarik et al. 2008, Ritz et al. unpublished). We assume this *canina*-nrITS type to be a trace for the existence of the hypothetic Protocaninae genome. The diploid Protocaninae are probably extinct but rescued as the bivalent forming set during meiosis (Fig. 2.2). On the other hand, the canina genome could also have evolved by mutation as it was shown in the evolution from teosinte to mayze in which also the existence of a “Proto-mayze” has been proposed (Doebley 2004). A polyphyletic origin of dog roses seems improbable keeping the uniqueness and complexity of canina meiosis in mind. However, phylogenetic trees based on chloroplast DNA sequences are polyphyletic with respect to the section *Caninae* because members of subsect. *Caninae* are not sister to the glandular species of subsect. *Rubigineae*

H. Christ and *Vestitae* H. Christ but to non-dog roses of sections *Indicae* Thory, *Rosa* and *Synstylae* DC. (Wissemann & Ritz 2005, Bruneau et al. 2007)

## 2.3 Character inheritance in the heterogamous system of dog roses

As a consequence of the canina-meiosis the proportion of genetic information contributed by the maternal parent to the offspring is four times larger than that of the pollen parent. Thus, offspring is largely matroclinal with respect to most morphological characters of leaves, flowers and hips (reviewed in Wissemann & Ritz 2007). This pattern of character inheritance is also expressed at the anatomical, biochemical and genetic level, since studies on epicuticular waxes and flower volatiles (Wissemann 2000a, Wissemann et al. 2007, Wissemann & Degenhardt, unpublished) and on microsatellites and random amplified polymorphic DNA (RAPD) bands (Werlemark 1999, Werlemark & Nybom 2001, Nybom et al. 2004, 2006, Ritz & Wissemann, submitted) demonstrated a strong matrocliny of interspecific hybrids.

However, some morphological traits do not match these observations, because Wissemann et al. (2006) showed that the growth habit of dog roses is dominantly inherited. Interspecific reciprocal hybrids between *R. canina* L. and *R. rubiginosa* L. were characterized by the lax and arching branches in contrast to dense erect branches of *R. rubiginosa*. This pattern could be the result of i) a heterosis effect, ii) the inheritance of dominant allele encoding growth form, or iii) which is favoured by the authors, multiple factors of which some are not inevitably subjected to inheritance but are responsible for the syndrome growth form.

Moreover, the taxonomic important characters sepal persistence and the diameter of the orifice are paternally expressed and possibly controlled by genomic imprinting (Gustafsson 1944, Ritz & Wissemann 2003). The paternal inheritance of these characters results in the morphological identity between interspecific hybrids and described dog rose species (Fig. 2.3). Ritz and Wissemann (submitted) demonstrated that *R. micrantha* Borrer ex Sm. and the corresponding interspecific hybrid *R. rubiginosa* × *R. canina* are genetically not completely identical.

Microsatellite alleles of *R. micrantha* corresponded to those of the potential parents, however, all investigated samples of *R. micrantha* were in contrast to the pentaploid hybrids hexaploid. The authors assumed that the initial interspecific hybrid giving rise to *R. micrantha* was established by an increase of ploidy level to maintain two highly homologous chromosome sets for correct bivalent formation during canina meiosis.

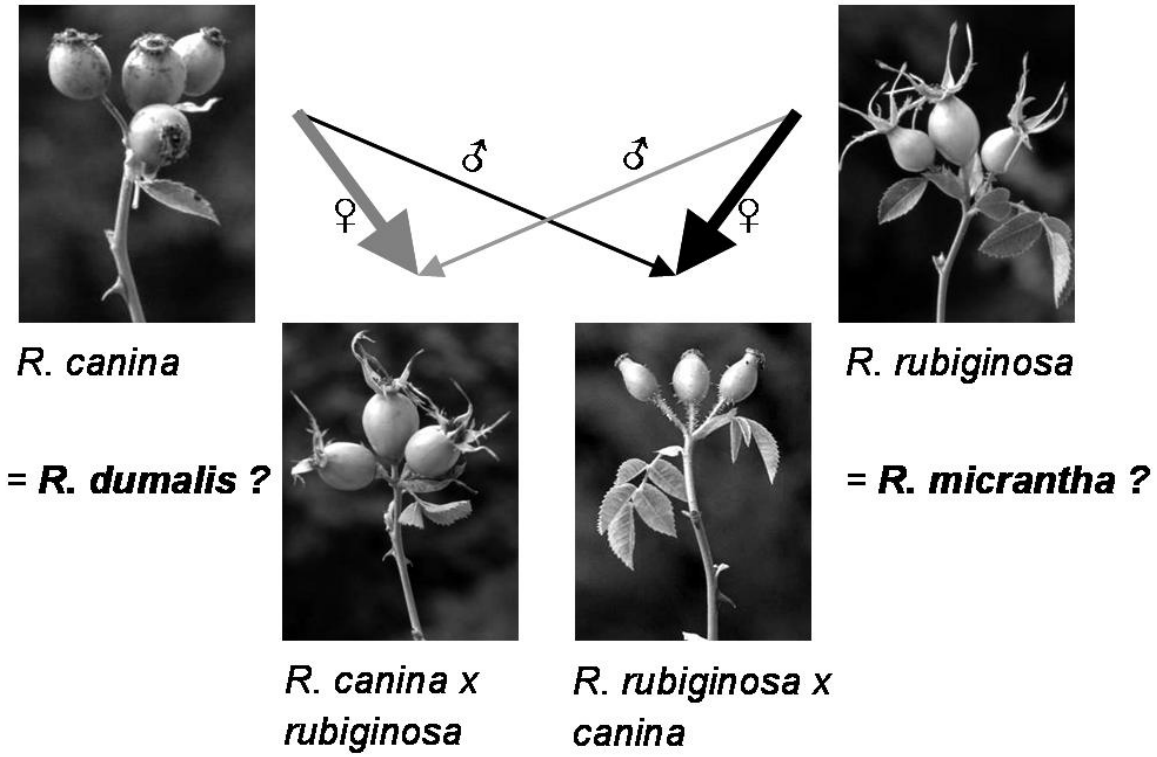


Fig. 2.3. Diagram of the crossing experiment (Wissemann & Hellwig 1997) between the two dog rose species *R. canina* (L-type: deciduous sepals during hip ripening) and *R. rubiginosa* (D-type: persistent sepals during hip ripening). Both parents were used as seed and pollen parent. The arrows symbolize the direction of the crosses. Grey arrows: *R. canina* was used as seed parent and *R. rubiginosa* was used as the pollen parent. Black arrows: *R. rubiginosa* was used as seed parent and *R. canina* as pollen parent. The thickness of the arrows symbolizes the matroclinal inheritance due to the canina meiosis: The seed parent inherits 4/5 of the genome and the pollen parent only 1/5 of the genome. The outcomes of both crosses show the same type of sepal persistence as the pollen parent. The combination of the matroclinal vegetative characters (e.g. leaf surface) and the paternal type of sepal persistence is also found in already described species (*R. dumalis* and *R. micrantha*) (Reproduction from Wissemann and Ritz (2007), Fig. 2)

The matrocliny of the majority of characters points despite the functional diploidy of the meiosis system to the functionality of genetic information stored on univalent genomes. Contrary, Lim et al. (2005) assumed that the lacking recombination between the univalents leads to gene degradation due to a relaxed selection pressure. However, a study on gene expression of single copy genes did not point to degradation or silencing of alleles on univalents (Ritz et al, unpublished).

## 2.4 Glandular trichomes matter: Rust fungi on *Rosa*

In Rosaceae, rust fungi have long been recognized as important parasites. Co-evolution of rusts and Rosaceae seems so strong and evident, that rust fungi can be used to determine phylogenetic relationships between certain hosts in Rosaceae (Savile 1979). El-Gazzar (1981) pointed out, that more than 1500 species from 49 genera of Rosaceae are susceptible to about 300 species out of 27 genera of the Uredinales, and that susceptibility to rust infection is strongly correlated with the chromosome base number of x=7. The most important rust fungi on *Rosa* are *Phragmidium mucronatum* and *Phragmidium tuberculatum*. Both have been recorded in dog roses (Gäumann 1959, Scholler 1994, Brandenburger 1994). Comparable to the findings of Evans et al. (2000) who showed the formation of subspecific strains or races of *Phragmidium violaceum* (Schultz) Winter, a parasite on the blackberry (*Rubus* L., Rosaceae), we expected the two *Phragmidium* species on roses to evolve and radiate in the same manner. However, ever since Savile's publication (1979) in which the interaction between rust fungi and Rosaceae has been regarded as a co-evolutionary system, our findings weakened this assumption (Ritz et al. 2005a). Host ranges of *P. mucronatum* and *P. tuberculatum* overlapped and their infection rates did not differ between *Rosa canina*, *R. corymbifera* Borkh. and *R. rubiginosa.* These three species served as examples for the variation of leaf trichomes and glands observed in dog roses which might be crucial for rust infection (Bahçecioğlu & Yildiz 2005, Valkama et al. 2005). Results showed that infection by *P. mucronatum* and *P. tuberculatum* did not significantly differ between species with glabrous and hairy leaves, *R. canina* and *R. corymbifera*, respectively. However *R. rubiginosa* developing hairy leaves with numerous odorous glands, was significantly less infected by both species (Ritz et al. 2005a). Despite their overlapping host ranges and their morphological similarity, both fungi are genetically only distantly related: *P. mucronatum* belongs to a clade of "rose rust sensu stricto" whereas *P. tuberculatum* is closely related to rusts living on *Rubus* and *Sanguisorba* L. and thus explored dog roses by a host jump. The lacking host specificity of rusts on dog roses might be explained by the hybrid bridge hypothesis (Floate & Whitham 1993). It predicts that hybridisation of hosts and thus the admixture of different genomes prevents the step-by-step process of co-evolution and co-speciation of hosts and parasites.

## 2.5 Evolution and diversity of plant-pathogen-insect foodwebs on dog roses

Herbivorous insect species and their host plant species together comprise more than 50% of the macroscopic species (Strong et al. 1984). Thus, an understanding of the evolutionary driving forces as well as the ecological interactions is an important issue for our general

understanding of biodiversity. During the last 30 years the paradigm of interpreting the enigmatic diversity of insects changed from co-evolution and co-speciation to a more individualistic view (e.g. Brandl et al. 1992, Schoonhoven et al. 1998).

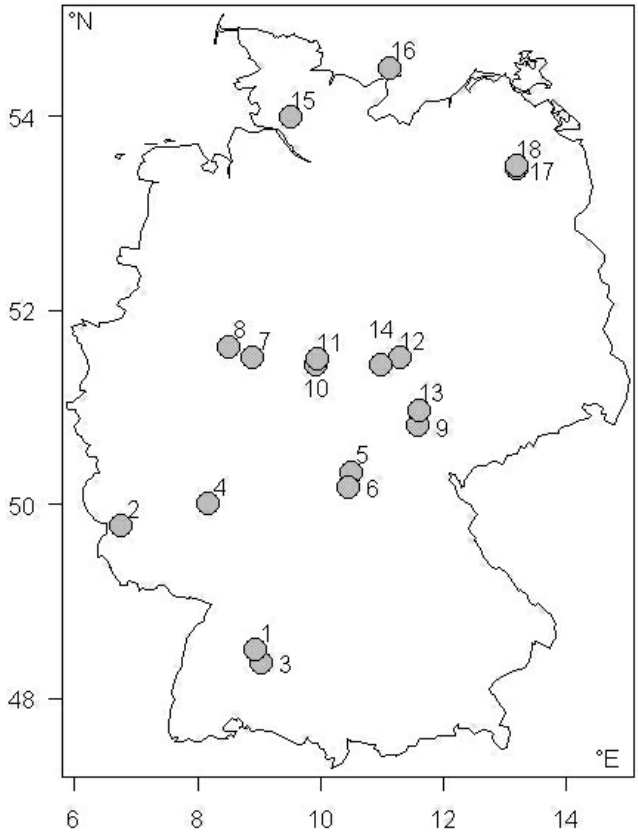


Fig. 2.4. Geographical location of the 18 study sites along a transect across Germany. At all study sites the three dog rose species (*Rosa canina, R. corymbifera, and R. rubiginosa*) occurred together. Community study was conducted at all 18 sample sites, density analyses of *Rhagoletis alternata* and *Diplolepis rosae* at 17 study sites (all except no. 10), and the *D. rosae* gall community was sampled on eight sites (No. 1-3, 5-6, 9, 11, 18).

Macroecological work of the last twenty years showed that the insect fauna associated with a particular plant species is a complex blend of generalists and specialists (Strong et al. 1984, Tscharntke & Greiler 1995, Schoonhoven et al. 1998, Brändle & Brandl 2001). The insect fauna on a particular host depends on the available species pool of phyto-phags, the distribution and abun-dance of the host, the number of feeding niches provided by the host as well as the host's taxonomic isolation and biochemical make-up (Strong et al. 1984, Lawton 1986, Tscharntke & Greiler 1995, Frenzel & Brandl 1998). Plant genotypes with different morphological traits may affect not only the abundance of single insect species but also the structure of associated herbivore communities (Maddox & Root 1987, Fritz & Price 1988). Whitham et al. (2003) showed that even single traits coded by few genes may have important effects on the community of exploiters (extended phenotype).

Rose bushes are characteristic features of European landscapes, in particular as components of hedges. Roses have been included into several attempts to understand $\alpha$-diversity (e.g. Leather 1986). However, most of the available data on phytophages have not distinguished between the different rose species and thus ignored the bewildering diversity within that host taxon. As roses provide a fascinating example of an explosive radiation, roses are good candidates to study the effects of hybridisation as well as rapid radiation of hosts on herbivores across a flock of host plants. The available information on phytophages

on roses shows that there are a number of generalists attacking roses (e.g. see Zwölfer et al. 1981, Zwölfer et al. 1984), but also many specialists such as the cynipid wasp *Diplolepis rosae* L., the tephritid fly *Rhagoletis alternata* Fallén, and the tortricid moth *Notocelia roborana* Dennis & Schiffermüller 1775. Ferrari for example sampled 6,000 ectophagous insect specimens from 32 rose stands around Göttingen (see Ferrari et al. 1997). Eight out of the ten tenthredinid wasps, three out of 19 Cicadina species, and four out of 76 beetle species were specialists on roses, whereas all the 20 bug species were less specialised.

For all our investigations we selected three dog rose species (*Rosa canina* L., *R. corymbifera* Borkh. and *R. rubiginosa* L.), all members of the dog rose section *Caninae* (DC.) Ser. These three species are widely distributed and abundant in central Europe and occur often in the same habitats. They are supposed to have originated by allopolyploid hybridisation events (Ritz et al. 2005b, Wissemann 2002) and expanded their range to central and northern Europe after the last ice age (Zielinski 1985). Although closely related they differ in several characters: *R. canina* is a glabrous rose, *R. corymbifera* has hairs on rhachis and abaxial leaf surface and *R. rubiginosa* has glandular trichomes on the lower leaf surface. Furthermore, the three rose species also differ in plant architecture (Wissemann et al. 2006) and phenology (Timmermann 1998). This leads to the question: How do these differences translate into the diversity of higher trophic levels? In the following we compare (1) the community structure of these three dog rose species, (2) the densities of two consumer specialists on the rose species and (3) the genetic adaptation of one of these specialists to the three dog rose species. Along a gradient across Germany (Fig. 2.4) we sampled at 18 study sites, where the three rose species occurred together in the same habitat.

### *2.5.1 Are invertebrate communities affected by leaf trichome traits of hosts?*

As already noted, the European dog roses differ in a variety of morphological traits, which may influence the interactions with associated food webs. In particular, dog roses differ considerably across species in density and type of trichomes on the lower leaf surface. Trichomes are supposed to influence host choice of herbivores as well as of other invertebrates (Yencho & Tingey 1994, Zvereva et al. 1998, Ranger & Hower 2002). Although trichomes are often part of a defence system, they may, however, be beneficial for some herbivores (Eisner et al. 1998). Nevertheless, invertebrate communities may map the variation of trichomes across species (Andres & Connor 2003).

Insect communities were sampled using beating trays (diameter 70 cm, Stechmann et al. 1981) in May, June, July and August 2002 (Fricke 2004). Data were collected across 88 bushes of *R. rubiginosa*, 87 of *R. corymbifera* and 88 of *R. canina* (3-5 bushes per sampling site, Fig. 2.4). We sorted 75,937 individuals to insect orders (Table 2.1). Coleoptera and

Heteroptera were identified to species level to distinguish between phytophagous and non-phytophagous species. To characterise the architecture of the sampled host individuals, six variables of each bush were measured.

With a repeated measurement ANOVA we found only minor differences in the abundance of common invertebrate groups (Aphidina, Collembola, Araneae, Hymenoptera and Coleoptera) between the three rose species (Table 2.1 and 2.2). Ordinations using all major taxonomic units showed also few differences in the composition of the exploiter communities. Contrary to our expectations, the abundances of phytophagous invertebrates were higher on *R. rubiginosa* than on the other two rose species. This is remarkable, because feeding experiments showed a lower palatability of *R. rubiginosa* leaves using larvae of *Spodoptera littoralis* (Klinge 2005; Fig. 2.5). Nevertheless, the field data suggest that most of the phytophagous invertebrates are not negatively affected by glandular trichomes and these trichomes do not serve as a general defence against phytophages.

Apart from phytophagous invertebrates, dog roses suffer from infection by the rust fungi *Phragmidium* spec. which shows significant differences in density between the three rose species (Ritz et al. 2005a, Klinge 2005, Fig. 2.6). The species *R. corymbifera* and *R. canina* show higher infection rates than the glandular *R. rubiginosa* (see Chapter 2.5). By infection with these rust fungi, the lower leaf surface of the host plant is of special concern. The rust fungi infect plants by penetrating the stomata of the leaflets with the germination tubes of its uredospores. The trichomes of dog roses occur only on the lower leaf surface were also the stomata are located.

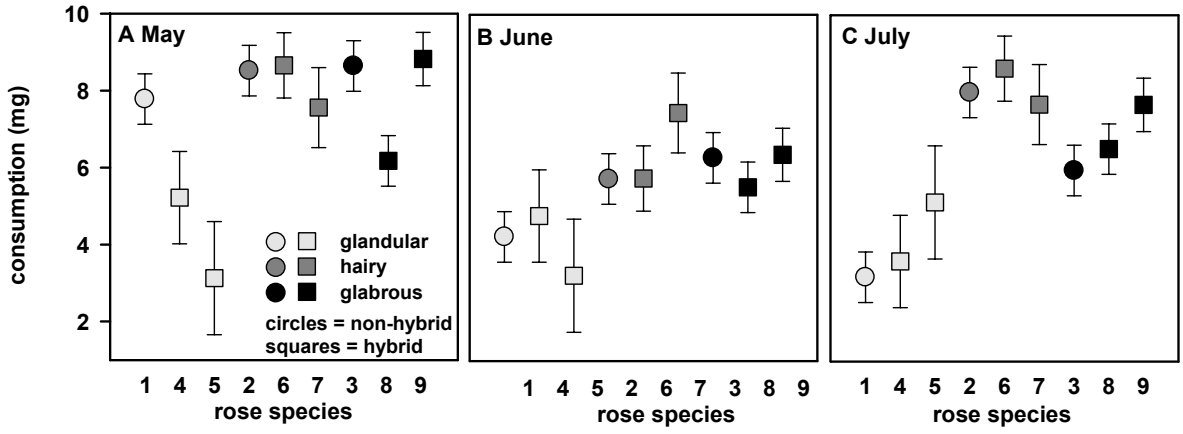


Fig. 2.5. *Spodoptera* leaf consumption across rose species and hybrids during three months. Corrected means ± 1 SE from a Split-Plot-Model ANCOVA (Type I) with leaf consumption as response variable in relation to the two categorical factors (month of experiment and rose genotype) and the covariables (fresh weight of larvae and specific water content). (rose species: 1 = *R. rubiginosa*, 2 = *R. corymbifera*, 3 = *R. canina*, 4 = *R. rubiginosa* x *R. corymbifera*, 5 = *R. rubiginosa* x *R. canina*, 6 = *R. corymbifera* x *R. rubiginosa*, 7 = *R. corymbifera* x *R. canina*, 8 = *R. canina* x *R. rubiginosa*, 9 = *R. canina* x *R. corymbifera*).

Table 2.1. Basic information for the invertebrate groups sampled from 263 shrubs of roses during May, June, and July 2002 across 18 sites in Germany (see Fig. 2.4). Taxa were sorted by the total number of individuals (N). The first five groups (names in bold) were used for detailed analyses of the abundances. Mean: average abundance across one subsample (5 subsamples per bush). Abundance was measured as the number of individuals divided by the subsamples. Correlations of abundances (means across dates of standardised transformed abundances for each bush, n = 263) with scores of the first two principle components (PCA1 and PCA2) characterizing plant architecture. Significant correlations in bold. The principal components analysis (PCA) was performed with six measured variables of each bush. Two components passed the Kaiser criterion. The first component represented the size (PCA 1; eigenvalue: 2.31, explained variance: 38.6 %) and the second the form of an individual bush (PCA 2; eigenvalue: 1.31, explained variance: 21.8 %).

| Taxon | N | Mean | s.d. | PCA 1 | | PCA 2 | |
|---|---|---|---|---|---|---|---|
| | | | | r | p | r | p |
| Aphidina | 19,656 | 4.97 | 12.44 | -0.07 | 0.293 | 0.11 | 0.084 |
| **Collembola** | 19,140 | 4.97 | 8.21 | -0.01 | 0.866 | **0.16** | **< 0.05** |
| **Araneae** | 11,275 | 2.90 | 3.65 | **-0.36** | **< 0.001** | **0.19** | **< 0.01** |
| **Hymenoptera** | 5,842 | 1.50 | 2.32 | **-0.23** | **< 0.001** | **0.28** | **< 0.001** |
| Coleoptera | 3,714 | | | | | | |
| **herbivorous** | 2,648 | 0.67 | 1.56 | **-0.18** | **< 0.01** | 0.02 | 0.759 |
| other | 1,066 | 0.27 | 0.54 | **-0.37** | **< 0.001** | 0.11 | 0.081 |
| Thysanoptera | 2,969 | 0.74 | 2.00 | **-0.20** | **< 0.001** | **0.18** | **< 0.01** |
| Acari | 2,642 | 0.68 | 1.55 | 0.02 | 0.780 | 0.01 | 0.902 |
| Diptera | 2,481 | 0.63 | 1.01 | **-0.22** | **< 0.001** | 0.08 | 0.208 |
| Auchenorrhyncha | 2,259 | 0.59 | 1.81 | 0.02 | 0.796 | -0.00 | 0.970 |
| Heteroptera | 1,504 | | | | | | |
| herbivorous | 803 | 0.21 | 0.24 | 0.09 | 0.164 | -0.09 | 0.155 |
| carnivorous | 701 | 0.18 | 0.31 | 0.03 | 0.598 | 0.06 | 0.339 |
| Dermaptera | 1,359 | 0.35 | 0.65 | **-0.19** | **< 0.01** | -0.04 | 0.549 |
| Psocoptera | 1,147 | 0.29 | 0.58 | -0.06 | 0.315 | 0.09 | 0.154 |
| Lepidoptera | 814 | 0.21 | 0.38 | -0.06 | 0.340 | 0.02 | 0.775 |
| Gastropoda | 337 | 0.09 | 0.28 | 0.09 | 0.142 | 0.03 | 0.646 |
| Planipennia | 311 | 0.08 | 0.18 | -0.09 | 0.160 | 0.08 | 0.183 |
| Opiliones | 126 | 0.03 | 0.15 | -0.06 | 0.333 | -0.06 | 0.363 |
| Orthoptera | 59 | 0.02 | 0.07 | -0.06 | 0.367 | -0.06 | 0.370 |
| **Σ** | **75,937** | | | | | | |

Table 2.2. Repeated measures ANOVA (type I sums of squares) for the five most abundant invertebrate groups on roses (Table 2.1). The site was a random factor, while rose species and date were fixed factors. Contrast I was defined as *R. corymbifera* vs. *R. canina*, contrast II was defined as *R. rubiginosa* vs. *R. corymbifera* and *R. canina*. In the part above the horizontal line, the means of each shrub over the dates were used, in the part below the line, all samples were used. Significant effects are highlighted in bold.

| Source of variation | Aphidina | | Collembola | | Araneae | | Hymenoptera | | phyto. Coleoptera | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SS. | F | SS. | F | SS. | F | SS. | F | SS. | F |
| Foliage cover | 0.33 | 3.0 | 5.99 | **54.3** | 3.22 | **86.3** | 1.54 | **38.3** | 0.04 | 1.8 |
| PCA 1 | 0.12 | 1.1 | 0.29 | 2.6 | 2.37 | **63.5** | 0.50 | **12.4** | 0.39 | **16.0** |
| PCA 2 | 0.42 | **3.9** | 0.13 | 1.2 | 0.29 | **7.7** | 0.76 | **18.9** | 0.00 | 0.1 |
| Site | 14.35 | **7.8** | 26.20 | **14.0** | 12.72 | **20.1** | 4.56 | **6.7** | 4.87 | **11.9** |
| Rose species | 4.69 | **7.1** | 0.23 | 0.4 | 0.21 | 1.7 | 0.17 | 1.0 | 0.00 | 0.0 |
| Contrast I | 0.11 | 0.6 | 0.25 | 1.2 | 0.00 | 0.0 | 0.01 | 0.4 | 0.00 | 0.0 |
| Contrast II | 4.60 | **9.9** | 0.01 | 0.0 | 0.21 | 3.4 | 0.16 | 1.2 | 0.00 | 0.0 |
| Site x rose species | 11.25 | **3.0** | 10.17 | **2.7** | 2.15 | **1.7** | 2.93 | **2.1** | 2.28 | **2.8** |
| Site x contrast I | 3.24 | **1.8** | 3.71 | **2.1** | 1.06 | 1.7 | 0.66 | 1.0 | 0.52 | 1.6 |
| Site x contrast II | 7.92 | **4.0** | 6.19 | **3.0** | 1.04 | 1.6 | 2.29 | **3.4** | 1.76 | **4.2** |
| Residuals I | 22.42 | | 22.72 | | 7.68 | | 8.29 | | 4.96 | |
| Residuals I (contr I) | 14.20 | | 14.49 | | 5.09 | | 5.28 | | 2.59 | |
| Residuals I (contr II) | 25.85 | | 26.92 | | 8.79 | | 8.95 | | 5.48 | |
| Date | 14.74 | **11.1** | 31.18 | **22.2** | 21.83 | **87.7** | 5.68 | **14.4** | 4.36 | **17.8** |
| Date x foliage cover | 2.21 | **15.1** | 1.44 | **12.8** | 0.20 | **5.1** | 1.71 | **35.0** | 0.67 | **20.8** |
| Date x PCA 1 | 0.17 | 1.2 | 0.03 | 0.3 | 0.04 | 0.9 | 0.74 | **15.1** | 0.10 | 3.0 |
| Date x PCA 2 | 0.02 | 0.2 | 0.36 | **3.2** | 0.22 | **5.7** | 0.14 | 2.8 | 0.12 | **3.8** |
| Date x site | 22.54 | **9.0** | 23.90 | **12.5** | 4.23 | **6.3** | 6.72 | **8.1** | 4.17 | **7.6** |
| Date x rose species | 2.95 | **5.2** | 0.32 | 0.6 | 0.44 | **4.8** | 1.01 | **4.8** | 0.65 | **5.1** |
| Date x contrast I | 0.22 | 1.5 | 0.00 | 0.0 | 0.07 | 2.1 | 0.08 | 1.4 | 0.06 | 1.8 |
| Date x contrast II | 2.75 | **6.5** | 0.31 | 1.3 | 0.37 | **6.5** | 0.95 | **6.0** | 0.59 | **6.2** |
| Date x site x species | 9.65 | **1.9** | 8.57 | **2.2** | 1.56 | 1.2 | 3.62 | **2.2** | 2.15 | **2.0** |
| Date x site x contr I | 2.54 | 1.0 | 4.38 | **2.2** | 0.58 | 0.9 | 0.95 | 1.2 | 0.56 | 1.2 |
| Date x site x contr II | 7.17 | **2.9** | 4.17 | **2.0** | 0.96 | 1.4 | 2.67 | **3.2** | 1.60 | **2.9** |
| Residuals II | 30.27 | | 23.23 | | 8.19 | | 10.09 | | 6.63 | |
| Residuals II (contr I) | 21.08 | | 15.93 | | 5.13 | | 6.39 | | 3.82 | |
| Residuals II (contr II) | 32.95 | | 27.64 | | 8.87 | | 11.10 | | 7.25 | |
| **Total** | 136.14 | | 154.77 | | 65.35 | | 38.37 | | 31.39 | |

Thus, the trichomes might serve as a defence against rust infections. This idea is not new and was already proposed for other plant species by Bahçecioğlu & Yildiz (2005) and Valkama et al. (2005). Furthermore, in the glandular trichomes of *R. rubiginosa* secondary compounds belonging to the sesquiterpenes occur (Klinge, unpublished). These substances are known to inhibit the growth of fungi (e.g. Alvarez-Castellanos et al. 2001, Cakir et al. 2004). Overall our data do not support the hypothesis that trichomes evolved as a defence strategy against inverte-brates and especially against herbivores. How-ever, our data are consistent with the hypotheses that trichomes are a defence strategy against rust fungi.

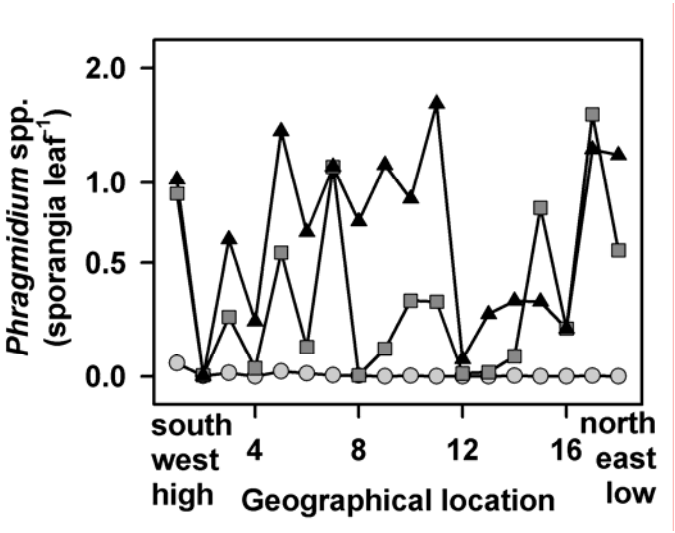


Fig. 2.6. Density of *Phragmidium spp.* across the three dog rose species (grey circle = *Rosa rubiginosa*, dark-grey square = *R. corymbifera*, black triangle = *R. canina*) and the geographical locations in the year 2002. Corrected means were calculated with General Linear Model analyses. Geographical location = ranking of the 18 sites from South to North, from West to East and from high to low altitudes based on results from a principal component analysis.

### *2.5.2 Do* Rhagoletis alternata *and* Diplolepis rosae *differ in density between the three rose species?*

Two highly specialised consumer species of dog roses are the European rose-hip fly *Rhagoletis alternata* Fall. (Diptera, Tephritidae) and the rose gall wasp *Diplolepis rosae* L. (Hymenoptera, Cynipoidea). The fruit fly *Rh. alternata* infests the fleshy fruits of species from several *Rosa* sections (White 1988). Adults emerge in early summer and females oviposit into green hips marking the attacked hips by an oviposition-deterring pheromone (Bauer 1986). Larvae feed exclusively in the hypanthium and do not attack the seeds. Mature larvae leave the hips for pupation and hibernate in the soil (Bush 1992). The percentage of infested hips per shrub is usually high, frequently reaching 100% (Bauer 1998). Overall, *Rh. alternata* has little impact on the sexual reproduction of roses and thus on the fitness of the hosts (Bauer 1998).

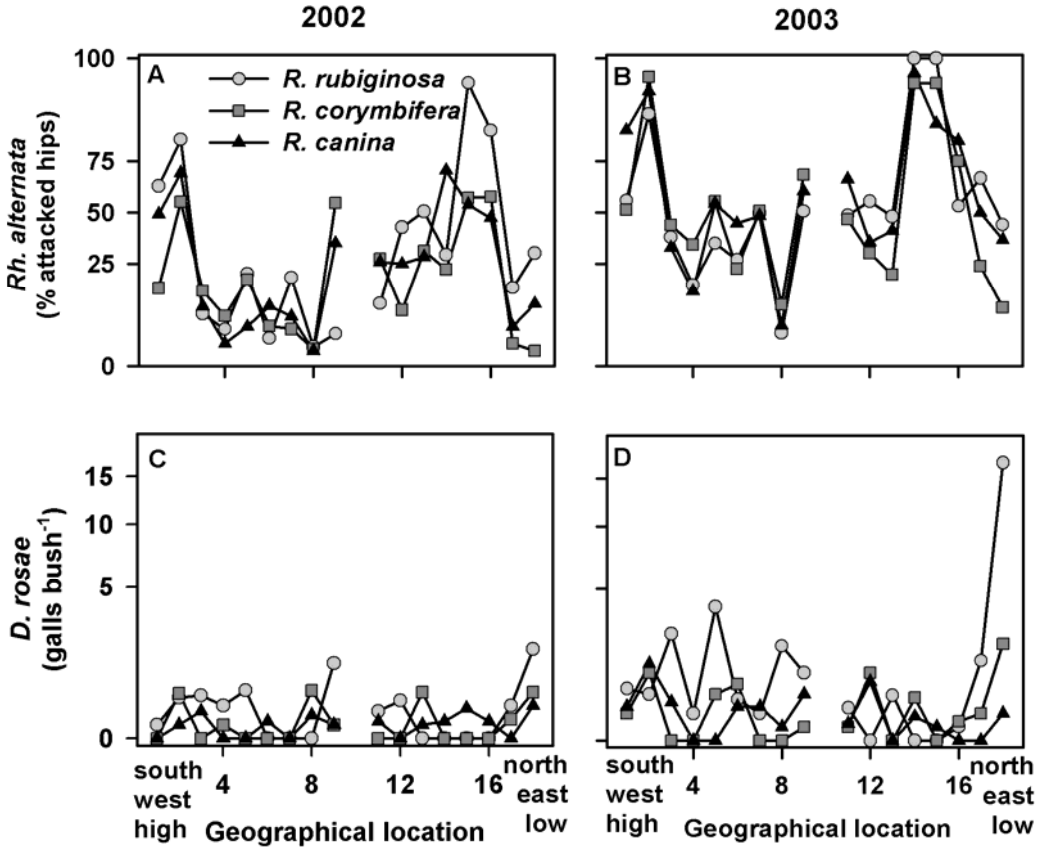


Fig. 2.7. Patterns of density across the three dog rose species and the geographical locations in the two study years 2002 and 2003 for (A, B) *Rhagoletis alternata* and (C, D) *Diplolepis rosae* (corrected means were calculated with General Linear Model analyses). Geographical location = ranking of the 18 sites from South to North, from West to East and from high to low altitudes based on results from a principal component analysis.

The holarctic cynipid wasp *D. rosae* is a univoltine gall maker (Adler 1877). In Europe, the conspicuous and multichambered galls have been found on *Rosa* species from several sections (Schröder 1967). In southern Sweden Stille (1984) found all species on which *D. rosae* occur belonging to the section *Caninae*. Due to the physiological manipulation of the host plant, cynipid gall wasps are closely adapted to their host plants (e.g. Crawley & Long 1995, Kato & Hijii 1997). The relationship between plants and gall inducing insects are usually very specific, suggesting tight co-evolutionary processes (Hilker et al. 2002). Galls develop as a result of interactions between the inducing insect and plant, wherein the insect gain control and redirect the growth and physiology of attacked organs to the insects' advantage (Shorthouse et al. 2005b). Both consumer species are known to attack all the three rose species. However, do the consumer species show differences in density between the host species? Klinge (2005) monitored the density of larvae of *Rh. alternata* and *D. rosae*

galls for each rose species in September 2002 and 2003 on 3 to 5 randomly selected bushes within each sample site (Fig. 2.4). To monitor the density of *Rh. alternata* 50 hips from each bush were collected haphazardly and the percentage of infested hips was used as an estimate of density. During the same sampling dates, rose bushes were searched for galls of *D. rosae*. The total number of galls on each bush was used as a measure of gall density.

Densities of the two consumer species varied between host plant species and geographical location (Fig. 2.7, Table 2.3). The highest densities of the two phytophages were found on the odorant *R. rubiginosa*. Although the density of *Rh. alternata* and *D. rosae* exhibited significant variations between sites, we found no general geographical trends. Two suites of factors may account for these differences: ecological and genetic factors. Plant phenology (Hodkinson 1997) and abundance of natural enemies (Koptur 1985) may be such possible factors or genetic variation of the consumer species may also trigger variation in density.

On the sexual reproduction of the roses *Rh. alternata* had little impact. Even when larvae attacked all hips of a bush, we did not find a negative impact of *Rh. alternata* densities on reproduction (number of hips) and leaf cover in the following year. Furthermore, *Rh. alternata* density patterns across years were highly predictable: Host plants with a high attack in one year showed also a high attack in the following year ($r^2$ = 0.45, P < 0.05, N = 212). We interpret the predictable fruit production as further evidence that larvae of *Rh. alternata* have overall little impact on the fitness of roses.

In contrast to *Rh. alternata*, the gall-forming *D. rosae* manipulates the physiology of the rose bushes to produce the gall and gall tissue (Bronner 1992, Bayer 1992, Bagatto et al. 1996, Harper et al. 2004). Although significant top-down effects of gall wasps on the population dynamics of the hosts seem to be rare (e.g. Stone et al. 2002), there is evidence that high cynipid densities can negatively affect host plant growth (e.g. Crawley & Long 1995, Kato & Hijii 1997). Due to the low density of *D. rosae* galls, with a mean density of 0.17 galls per bush, the roses did not show a negative response: The density of hips as well as leaf cover was independent of the number of galls in the previous year (hip density: $r^2$ = 0.02, P = n.s., leaf cover: $r^2$ = 0.02, P = n.s.).

### *2.5.3 Does* Rhagoletis alternata *form host races on the three dog rose species?*

Phytophagous insects may adapt to host plants, thereby forming host races as a first step during sympatric speciation. Host races are sympatric but genetically differentiated populations of exploiters that use different host species (Drès & Mallet 2002).

Table 2.3. Repeated measures ANOVA (type I sums of squares) for the densities of *Rhagoletis alternata* and *Diplolepis rosae*. The site was a random factor, while host species (*Rosa canina*, *R. corymbifera* and *R. rubiginosa*) and year were fixed factors. Differences in shrub characteristics were accounted for with the two principal components (PC1 and PC2) derived in a PCA. PC1 characterizes the size of a shrub (35% explained variance; variables with loading > 0.6: height, diameter, diameter of the largest shoot) and PC2 the foliar density of a shrub (26% explained variance; loadings > 0.6: density of leaflets and hips, leaf cover). SS = sum of square, df = degrees of freedom; the F-ratio was calculated with the appropriate error term; significant effects are marked in bold.

| | *Rh. alternata* | | | *D. rosae* | | |
|---|---|---|---|---|---|---|
| | SS | df | F | SS | df | F |
| PC1 | 0.16 | 1 | 2.24 | 0.59 | 1 | 1.24 |
| PC2 | 0.20 | 1 | 2.71 | 16.93 | 1 | **35.61** |
| Site (S) | 38.73 | 16 | **33.63** | 34.33 | 16 | **4.51** |
| Host (H) | 1.15 | 2 | **3.69** | 12.86 | 2 | **6.01** |
| S x H | 4.98 | 32 | **2.16** | 34.21 | 32 | **2.25** |
| Residuals | 11.45 | 159 | | 82.26 | 173 | |
| Year (Y) | 10.77 | 1 | **48.09** | 10.11 | 1 | **18.14** |
| Y x PC1 | 0.18 | 1 | **5.99** | 0.02 | 1 | 0.06 |
| Y x PC2 | 0.18 | 1 | **6.21** | 4.61 | 1 | **17.45** |
| Y x S | 3.58 | 16 | **7.61** | 8.92 | 16 | **2.11** |
| Y x H | 0.29 | 2 | 2.01 | 2.54 | 2 | 1.89 |
| Y x S x H | 2.28 | 32 | **2.42** | 21.53 | 32 | **2.55** |
| Residuals | 4.68 | 159 | | 45.65 | 173 | |

Vaupel et al. (2007) studied the population genetic structure of *Rh. alternata* along the geographic gradient and in relation to the three rose species. They collected larvae from 15 sites across Germany and from three valleys of Valais in Switzerland. They were able to score nine allozyme loci (five polymorphic). Populations from the three hosts did not differ in genetic variability. These results provide two further unexpected findings. Firstly, although they found significant genetic differentiation between populations from different host species, the differentiation was very low (0.9%) and cannot be interpreted as an indication for host races. A reason may be the permanent and ongoing hybridisation between rose species of the section *Caninae*. Secondly, they found surprisingly little geographic structure of genetic differentiation between populations of this fruit fly across central Europe. Additionally, analysis of amplified and sequenced fragments of the mitochondrial genes encoding cytochrome oxidase I (800 bp), cytochrome oxidase II (470 bp), and cytochrome *b* (450 bp), indicated that all individuals of *Rh. alternata* (n = 21) from several sites in Europe shared the

same haplotype (Kohnen et al. 2009, see Chapter 6). This lack of genetic variation is unexpectedly low compared to data of other insect taxa ($p = 0.0016$, $n= 63$).

Three mutually non-exclusive reasons may explain these findings. Firstly, gene flow between populations of *Rh. alternata* is high. Secondly, the pattern of genetic differentiation is based on a recent expansion of the distributional range or a host shift of the fly. Thirdly, symbionts, such as *Wolbachia,* shape at least mtDNA evolution (Hurst & Jiggins 2005). During the initial phase of symbiont invasion, selective sweeps may reduce mtDNA diversity, thereby producing a genetic signal similar to that produced by a population bottleneck with subsequent expansion (Hurst & Jiggins 2005).

Because of the low gene flow estimated from allozyme data *Rh. alternata* seems to be a good disperser (Leclaire & Brandl 1994, Vaupel et al. 2007). Even the Alps do not seem to be a geographical barrier for gene flow between populations. In part this is explained by the behaviour of the females which mark the hips with a pheromone after oviposition (Bauer 1986, 1998). Often a high proportion of hips (up to 100%) are infested. Females leave such localities and search for rose shrubs with a lower proportion of infested hips. Vaupel et al. (2007) found no isolation by distance ($r^2 = 0.01$, $p = 0.19$), which may indicate that populations are not in an equilibrium between drift and gene flow (Hutchison & Tempelton 1999). The time to reach population genetic equilibrium increases with population size. The population size of tephritids is often very high (McPheron et al. 1988), and *Rh. alternata* is no exception. Low genetic differentiation and lack of isolation by distance points to a recent range expansion (Hutchison & Templeton 1999). Such an expansion could be induced by colonisation events after the last ice ages or by very recent host-shift events. In both cases, a low number of founder individuals would lead to a population bottleneck. Lower levels of genetic variation would be expected (Harrison 1991). *Rh. alternata* is a specialist on members of the genus *Rosa* section *Caninae* and therefore dependent on the distribution of its host. These dog roses originated by hybridisation events during the last ice ages (Ritz et al. 2005b, Wissemann 2002) and re-colonised Europe afterwards (Dingler 1907). Founder individuals of the fly species may have shifted to this new host, providing an explanation for the low genetic variability. Overall the considerable gene flow between populations of *Rh. alternata*, limited phenological differences between host species, and the ongoing hybridisation of hosts may prevent the formation of genetic differences between populations of exploiters on rose species.

## 2.6 How are the differences between the three closely related dog rose species translated into higher trophic levels?

Due to the enclosed environment within the galls, gall-makers and their parasitoids provide a good opportunity to analyse tritrophic interactions. Gall characteristics like shape and

toughness are plant derived structures, but often regulated by insect genes whereas the gall diameter for example is regulated by plant genotype (in *Salix lasiolepis*; Price & Clancy 1986b). In turn the galls sizes and densities determine the success as well as composition of parasitoid communities (Brandl & Vidal 1987, Schlumprecht 1989, Weis 1983).

So far, we showed certain differences in community structure and consumer density between the glandular rose *R. rubiginosa* and the other two rose species. The galls of *D. rosae* form the basis of a complex community of an inquiline and at least 12 species of parasitoids (Blair 1944, Redfern & Askew 1992). Beside *D. rosae*, the inquiline *Periclistus brandtii* R. (Hym. Cynipidae) and several parasitoid wasp species can be found within the galls (Redfern & Askew 1992). The gall-maker *D. rosae* is parasitized by at least five parasitoid species: *Orthopelma mediator* Thunb. (Hym. Ichneumonidae), *Torymus bedeguaris* L. (Hym. Torymidae), *Pteromalus bedeguaris* Thomson (Hym. Pteromalidae), *Glyphomerus stigma* Fabr. (Hym. Torymidae) and *Eupelmus urozonus* Dalman (Hym. Eupelmidae). The dominant one which is almost invariably present is *O. mediator* (Stille 1984). The inquilin *P. brandtii* utilizes the galls to create its own chambers on the surface of the gall. The effect of *P. brandtii* attack on the gall is so far unknown, either it enlarges the gall or reduces the space otherwise available to *D. rosae*. The inquilin is also parasitized by *G. stigma* and *E. urozonus*, but additionally by *Caenacis inflexa* Ratzeburg (Hym. Pteromalidae) and *Eurytoma rosae* Nees (Hym. Eurytomidae).

We sampled all available *D. rosae* galls at eight of the sample sites (Fig. 2.4) and hatched the galls outside until all inhabitants emerged (Table 2.4). With a generalised linear model the parasitism rate of *D. rosae* varied between sites as well as rose species and decreased with increasing gall volume (Klinge 2005, see Chapter 3). Whereas the mean number of *D. rosae* galls was highest on *R. rubiginosa*, the mean parasitism rate was lowest on this rose species. But all two way interactions between host species and site were also significant, pointing to complex effects of geography and rose species on the communities associated with *D. rosae* galls. As mentioned above, *R. rubiginosa* is the only rose species we examined with glandular trichomes rich in secondary metabolites as sesquiterpenes. Host plant variation and differences in secondary metabolites often influence higher order interactions within insect communities (Eisenbach 1996, Fritz et al. 1997, Gange 1995, Marquis & Whelan 1996, Prezler & Boecklen 1994).

Beyond factors intrinsic to the host plant, local environmental conditions have also some influence on plant traits e.g. resulting in different nutritive quality and thereby changing interactions with herbivores and their natural enemies (Moon & Stiling 2000, Price & Clancy 1986b, Stiling & Rossi 1997). Even effects of genetic differences among and within host plant taxa may be modified by environmental conditions (Fritz et al. 1997).

Table 2.4. The inhabitants of *Diplolepis rosae* galls, based on rearings of n= 299 galls. Numbers of individuals are given for the gall-maker *D. rosae*, the inquilin *Periclistus brandtii* and the parasitoids (sum of individuals, mean ± standard error (back transformed values), the minimum and maximum). Parasitoids of *D. rosae* are marked with a "D" and parasitoids of *P. brandtii* with a "P".

| | host | sum of individuals | mean | -se | +se | Maximum per gall |
|---|---|---|---|---|---|---|
| *Diplolepis rosae* | | 1968 | 2.08 | 1.87 | 2.31 | 130 |
| *Periclistus brandtii* | | 765 | 0.54 | 0.45 | 0.62 | 73 |
| *Orthopelma mediator* | D | 1111 | 1.36 | 1.22 | 1.50 | 56 |
| *Glyphomerus stigma* | D,P | 751 | 1.14 | 1.03 | 1.26 | 28 |
| *Torymus bedeguaris* | D | 562 | 0.84 | 0.76 | 0.93 | 44 |
| *Pteromalus bedeguaris* | D | 377 | 0.67 | 0.61 | 0.74 | 24 |
| *Caenaxis inflexa* | P | 326 | 0.28 | 0.23 | 0.33 | 38 |
| *Eurytoma rosae* | P | 131 | 0.24 | 0.20 | 0.27 | 9 |
| *Eupelmus urozonus* | D,P | 105 | 0.18 | 0.15 | 0.21 | 20 |
| *Torymus rubi* | D | 32 | 0.06 | 0.05 | 0.08 | 4 |
| *Torymus* spp. | ? | 16 | 0.03 | 0.02 | 0.04 | 3 |

In the same way Thompson (2005) suggests in his Geographic Mosaic Theory of Coevolution, that interactions among species may be modified by environmental conditions. Thompson (2005) considers a dynamic process of varying species compositions, environmental conditions and following variations in interactions. Our data show significant variations in community structures and consumer densities between geographical sites. Environmental variations among sites did not only alter abundance of *D. rosae* but also structure and success of higher trophic levels, the associated community of parasitoids (Klinge 2005, see Chapter 3). During this study it was investigated how the phenotypic plasticity of dog roses affects next trophic levels. But interactions between the host plant species and herbivore communities changed depending not only on phenotypic differences between the host plant species but also on their geographical location.

## 2.7 Conclusion

The guiding question of our study was how the radiation and diversity of the hosts translate into the radiation and diversity of the higher trophic levels and what role interactions play in

the whole system? We observed a major impact of the *Canina* breeding system on character evolution and thus influencing direct interaction between hosts and parasites. However, interactions between host plant species and herbivore communities changed depending not only on phenotypic differences between the host plant species but also on their geographical location. Thus, genetic diversity of dog roses is not translated into a host-specific radiation process of the parasites. We assume that the intensive reticulate evolution of dog roses via hybridisation prevents co-speciation.

## ACKNOWLEDGEMETS

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# 3. Cynipid gall-parasitoid interactions, comparing three dog rose species along a geographical gradient

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## ABSTRACT

Multitrophic interactions between plants, herbivores and their natural enemies may change with host species as well as in a geographic context. Thus, the genetic variation between the hosts affects not only herbivores but also the structure and dynamics at higher trophic levels. Due to the enclosed environment within galls, gall-makers and their parasitoid community were used to analyse tritrophic interactions. We tested how the associated parasitoid community of the cynipid gall wasp *Diplolepis* rosae L. (Hym. Cynipidae) differed between three closely related rose species (*Rosa canina* L.*, R. corymbifera* Borkh. and *R. rubiginosa* L.) across a geographical gradient. Within 388 galls eight species of parasitoids (most common: *Orthopelma mediator* 18%, *Glyphomerus stigma* 12%, *Torymus bedeguaris* 9.1%) and the inquiline *Periclistus brandtii* (12%) were found. High variations in gall densities, as well as in parasitism rates at different geographical locations, were found. The environmental conditions also influenced the gall volume and the communities associated with *D. rosae* galls. However, the host species had only an indirect effect in either influencing the gall density by the shrub's habit or showing significant interactions with the geographical location. With increasing gall volume the rate of parasitism decreased due to the length of parasitoid's ovipositor. Overall, we conclude that the ongoing hybridisations between rose species prevent herbivores and their parasitoids establishing clear host preferences. Nevertheless, in combination with the geographical location the host species has an important impact on the abundance of exploiter densities and their dependent communities.

## 3.1 Introduction

The insect fauna on a particular host depends on the available species pool of phytophages, the distribution and abundance of the host, the number of feeding niches provided by the host as well as the host's taxonomic isolation and biochemical make-up (e.g. Fritz et al. 1997, Harvey et al. 2003, Cattell & Stiling 2004). It has been known for a long time, that genetically based plant traits including secondary compounds, plant architecture and nutritional value, vary among even closely related host plant species (e.g. Orians 2000, Pichersky & Gang 2000, Malmstrom et al. 2000). A number of studies showed that this genetic variation between hosts affects not only herbivorous insects but also the structure and dynamics of higher trophic levels and therefore whole communities (e.g. Bailey et al. 2006). Most of the studies used interspecific hybrids (Fritz et al. 1997, Hochwender & Fritz 2004). Fritz et al. (1994) postulated four models to describe the patterns of herbivores on hybrids, which are the susceptibility model (greater loads on hybrids), resistance model (lower load), additive model (intermediate loads), and the dominance model (herbivore load of one parent species).

But even intraspecific variation has some effect on the composition of herbivores (Rudgers & Whitney 2006, Espirito-Santo et al. 2007, Halverson et al. 2008). Here again, plant architecture has contrary effects on different herbivores, some were found more often on prostrate plants and others on erect plants (Rudgers & Whitney 2006). Depending on the composition of herbivores the community of natural enemies is variable. But not only the composition of herbivores, even the quality of herbivores, due to the food plants chemistry, results in differential development and fitness of higher trophic levels (e.g. Orr & Boethel 1986, Harvey et al. 2003, Ode et al. 2004, Setamou et al. 2005, Ode 2006). For instance some herbivores are able to sequester plant toxins in their own tissue for protection against their natural enemies (Wink et al. 2000, Müller et al. 2001). Allelochemicals in host or prey diet negatively affect the growth, development, survival and morphology of their predators or parasitoids (e.g. Harvey et al. 2003).

Beyond these factors intrinsic to the host plant, local environmental conditions have also some influence on plant traits e.g. resulting in different nutritive quality and thereby changing interactions with herbivores and their natural enemies (Price & Clancy 1986b, Stiling & Rossi 1997, Moon & Stiling 2000). Even effects of genetic differences among and within host plant taxa may be modified by environmental conditions (Fritz et al. 1997). In the same way Thompson (2005) suggests in his Geographic Mosaic Theory of Co-evolution that interactions among species may be modified by environmental conditions. Co-evolution should be an ongoing process shaped by the geographic structure and differing across geographical locations. Natural selection on interspecific interactions should vary among

geographical locations and among populations depending on the constitution and the fitness of the interacting species. Due to varying distributions of locally specialised genotypes differences in fitness of the dependent species should occur. Interacting species in different populations should converge on different combinations of traits and even on different combinations of partners. The pattern of interactions therefore should be a mosaic between geographical locations.

One major group of herbivorous insects are the gall-forming insects which have a specialised relationship to their host plants. Gallers induce the development of modified host tissues with high nutritive value and very low concentrations of toxic secondary plant metabolites (e.g. Hartley 1998, Stone & Schönrogge 2003). Due to the enclosed environment within the galls, gall-makers and their parasitoids provide a good opportunity to analyse trophic interactions. The tri-trophic interactions of the gall forming cynipid wasp *Diplolepis rosae* L. (Hym.) at different geographical localities in Germany were examined. This gall wasp only attacks species of the genus *Rosa*. In Europe, the conspicuous and multichambered galls have been found especially on species of the section *Caninae* (DC.) Ser (Schröder 1967). We examined the trophic interactions on three rose species (*Rosa canina* L.*, R. corymbifera* Borkh. and *R. rubiginosa* L.), which are widely distributed and abundant in central Europe and often occur in the same habitats. They are supposed to have originated through allopolyploid hybridisation events (Wissemann 2002, Ritz et al. 2005b) and expanded their range to central and northern Europe after the last ice age (Zielinski 1985). Although closely related the three rose species differ in several characters e.g. in plant architecture (Wissemann et al. 2006) and phenology (Timmermann 1998). The galls of *D. rosae* form the basis of a complex community of an inquiline and at least 12 species of parasitoids (Blair 1944, Redfern & Askew 1992).

In the following we want to consider if differences of the closely related host plants alter densities and communities of this complex plant-gall-parasitoid interaction. In particular we pose the questions:

(1) Does the gall density vary between rose species at different geographical localities?
(2) Do gall characteristics depend on the rose plant species?
(3) Does the parasitism rate differ on the rose plant species?
(4) Does the gall-parasitoid community change with the rose plant species or the geographical location?

## 3.2 Material and Methods

*Gall community*

Beside *D. rosae*, the inquiline *Periclistus brandtii* R. (Hym. Cynipidae) and several parasitoid wasp species can be found within the galls (Redfern & Askew 1992). The gall-maker *D. rosae* is parasitised by at least five parasitoid species: *Orthopelma mediator* Thunb. (Hym. Ichneumonidae), *Torymus bedeguaris* L. (Hym. Torymidae), *Pteromalus bedeguaris* Thomson (Hym. Pteromalidae), *Glyphomerus stigma* Fabr. (Hym. Torymidae) and *Eupelmus urozonus* Dalman (Hym. Eupelmidae). The dominant one which is almost invariably present is *O. mediator* (Stille 1984). The inquilin *P. brandtii* utilizes the galls to create its own chambers on the surface of the gall. The effect of *P. brandtii* attack on the gall is so far unknown, either it enlarges the gall or reduces the space otherwise available to *D. rosae*. The inquilin is also parasitised by *G. stigma* and *E. urozonus*, and additionally by *Caenacis inflexa* Ratzeburg (Hym. Pteromalidae) and *Eurytoma rosae* Nees (Hym. Eurytomidae).

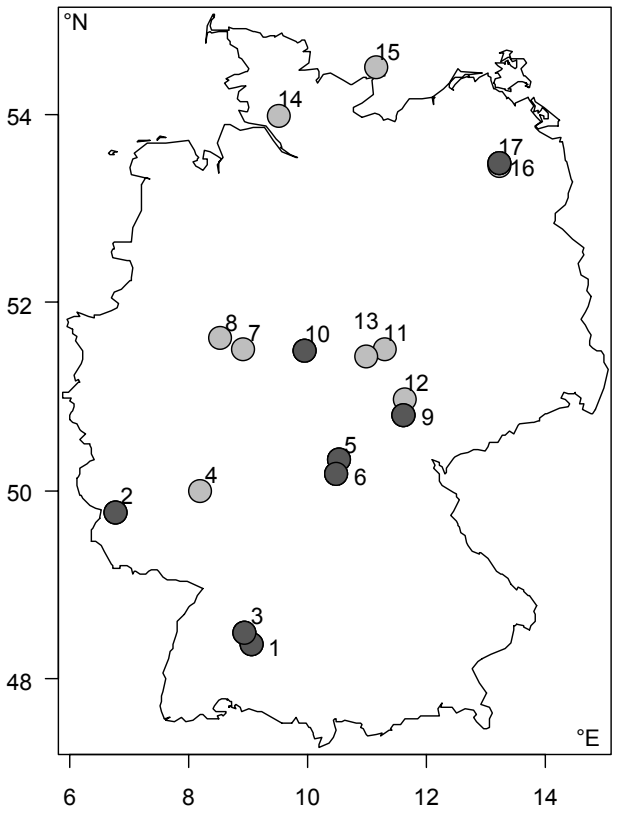



Fig. 3.1. Sample sites across Germany. At all 17 sample sites *Diplolepis rosae* gall densities were measured on five shrubs per rose species (*Rosa rubiginosa*, *R. corymbifera* and *R. canina)*. At eight sites (black) the gall parasitoid communities of all available galls were hatched.

*Field survey*

Galls of *D. rosae* on three rose species (*R. canina*, *R. corymbifera* and *R. rubiginosa*) at 17 localities across Germany during October 2002 and 2003 (Fig. 3.1) were counted and sampled. Longitude, latitude and altitude of each sample site were recorded using a Global Positioning System ("GPS 12 Personal Navigator", © Garmin International Inc.). We monitored all galls on 5 randomly selected shrubs within each sample site throughout 2002. The total number of galls on each shrub was used as a measure of gall

density. Since plant architecture may influence abundance of invertebrates, we collected data to characterise the architecture of the sampled shrubs. Shrub height (m) and diameter (m), number of shoots and diameter of the largest shoot (cm), density of hips and leaflets of the compound leaves were measured. Hip and leaflet density of a shrub was measured by counting hips and leaflets along 30 cm from the tip of five randomly selected branches, of which the mean was calculated. For subsequent analyses the variables were transformed to $\log_{10}$ (a one was added to the number of hips).

At eight of the 17 sample sites (Fig. 3.1) all existing galls for each rose species were sampled in 2003. The galls were kept individually outside, in plastic pots covered with gauze from October to July. Until July all inhabitants were allowed to emerge then the galls were dissected. Thus the number of chambers initiated by larvae of *D. rosae* as well as by larvae of inquilines could be established. The chambers of both species are distinguishable due to the larger size of *D. rosae* chambers (Redfern & Askew 1992). Width and depth of the galls were measured to estimate the gall volume by the formula of a spheroid (gall volume = 4/3 * 1/8 * width$^2$ * depth * π). All sampled individuals were identified to species level. For all subsequent analyses the gall volume was ln-transformed.

*Statistics*

To reduce the number of variables characterising the individual shrubs a PCA on the correlation matrix was performed. Prior to analyses the six variables were centralised and standardised by subtracting the mean and dividing by the standard deviation. Two principle components passed the Kaiser criterion with eigenvalues higher than one. PC1 characterizes the size of a shrub (42% explained variance, eigenvalue 2.5, variables with loading > 0.4: height, diameter, diameter of the largest shoot) and PC2 the habit of a shrub (24% explained variance, eigenvalue 1.42, loadings > 0.4: number of shoots, density of leaflets and hips).

We analysed the data using generalised linear models using “R” ver. 2.4.1 (R Development Core Team 2006), using the Quasipoisson distribution with log link function for count data and Quasibinomial distribution with logit link function for proportions. The sample site was considered as a random factor. Therefore the F-values of the fixed factor (rose species) were calculated with the appropriate df (interaction between site and rose species).

Firstly, the density of galls found on each shrub (galls of all 17 sampling sites, Fig. 3.1) were tested against the two principle components PC1 and PC2, characterising the shrub individuals, and two factors: the site and the rose species (glm, Quasipoisson distribution) with all two-way interactions.

Secondly, the gall volume (glm; galls of eight sampling sites, Fig. 3.1) and the number of inhabitants (glm, Quasipoisson distribution) were tested against sampling site and rose species, respectively, after prior correction of the gall volume for the number of inhabitants.

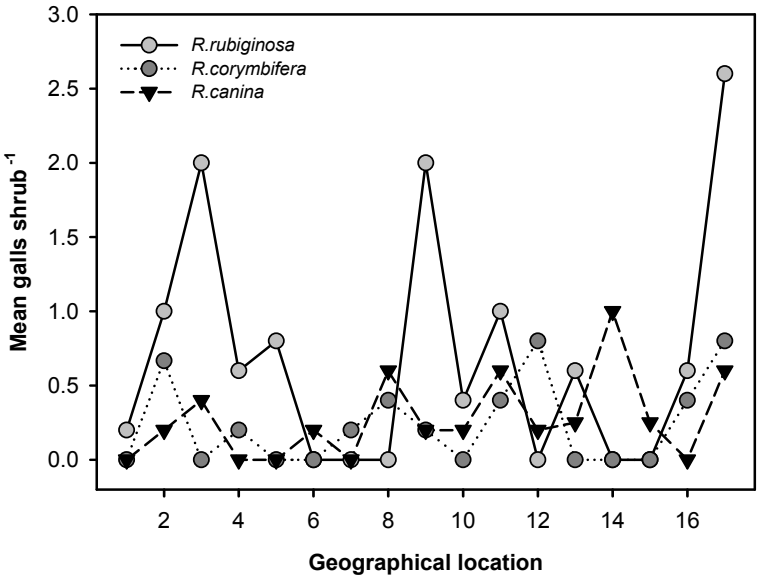



Fig 3.2. The mean number of *Diplolepis rosae* galls per shrub on three different host species *Rosa rubiginosa*, *R. corymbifera* and *R. canina* at 17 sites in Germany.

Thirdly, the percent of parasitism (galls of eight sampling sites, Fig. 3.1) was tested against the sampling site, the gall volume and the rose species (glm, Quasibinomial distribution) with all two-way interactions. The percent of parasitism was calculated for the *Diplolepis*-complex and the *Periclistus* complex, respectively. We included in the *Diplolepis*-complex all parasitoids of *D. rosae* themselves and species, which parasitise *D. rosae* as well as *P. brandtii* (*O. mediator*, *T. bedeguaris*, *P. bedeguaris*, *T. rubi*, *G. stigma* and *E. urozonus*, N = 292 galls). In the same way the percent parasitism within the *Periclistus*-complex was estimated (parasitoids are: *C. inflexa*, *E. rosae*, *G. stigma* and *E. urozonus*, N = 204 galls).

To test whether the abundance of the parasitoid community depends on the rose species, we performed a variation partitioning analysis using redundancy analysis ordination (RDA). All individuals, which where not specified to the species level (*Torymus* spp.) were excluded and all other species (N = 10) were square root transformed. Three explanatory tables consistent of (1) the gall volume ($log_{10}$ transformed), (2) the sampling site (longitude and latitude) and (3) the rose species (coding with two dummy variables) were included. To calculate the adjusted $R^2$ of the single fractions RDA methods with 200 permutations were used. Ordination methods were conducted using package “vegan” in “R” ver. 2.4.1 (R Development Core Team 2006).

## 3.3 Results

At the 17 sample sites the gall density differed widely between shrubs (Fig. 3.2). Some shrubs contained 10 galls, whereas on the majority (80%) no galls were found at all. The mean number of galls per shrub was 0.4, varying between rose species (*R. rubiginosa* 0.69; *R. corymbifera* 0.24 and *R. canina* 0.28). The abundance of galls shrub-1 depended significantly on PC2 (characterising the habit of the shrub), the site, and several interactions (Table 3.2), but was independent of the rose species. But the rose species differ in their characteristics (PC1, $F_{2,231} = 49.64$, $p<0.001$ as well as PC2, $F_{2,231} = 21.20$, $p<0.001$). *R. rubiginosa* is slightly taller and has a denser habit.

Table 3.1. Distribution and characteristics of the *Diplolspis rosae* galls on the three investigated rose species. Mean numbers and percent parasitism are given with standard errors. The inhabitants of *D. rosae* galls, based on a total of 298 galls, mean numbers gall$^{-1}$ are given for the gall-maker *D. rosae*, the inquilin *Periclistus brandtii* and the parasitoids (± standard error). Parasitoids of *D. rosae* are marked with a "D" and parasitoids of *P. brandtii* with a "P". The total number of all inhabitants is 6,175.

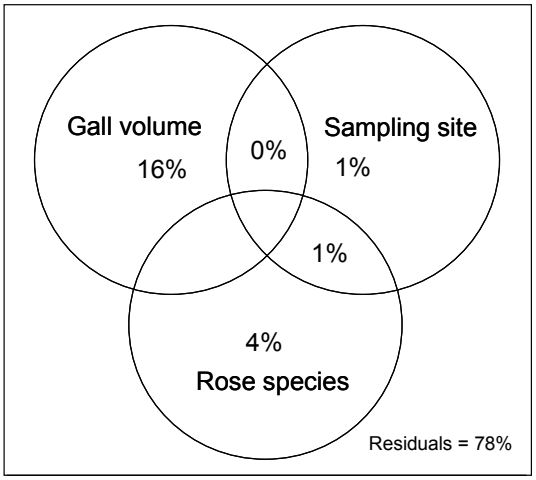
| | | *R. rubiginosa* | *R. corymbifera* | *R. canina* | N |
|---|---|---|---|---|---|
| No. of bushes | | 29 | 29 | 75 | 133 |
| No. of galls | | 170 | 80 | 133 | 383 |
| Mean No. galls/bush | | 5.86 ± 1.35 | 2.76 ± 0.52 | 1.77 ± 0.14 | 135 |
| Mean No. of Ind /gall | | 20.18 ± 2.78 | 16.63 ± 1.71 | 24.54 ± 2.43 | 298 |
| Gall volume ($cm^3$) | | 5.55 ± 0.64 | 6.56 ± 0.81 | 10.39 ± 1.38 | 386 |
| Species No. / gall | | 2.93 ± 0.14 | 3.43 ± 0.21 | 3.69 ± 0.21 | 289 |
| Mean Parasitism / gall (%) | | 62.75 ± 3.09 | 75.98 ± 3.10 | 75.66 ± 2.64 | 289 |
| Mean *Diplolepis rosae* / gall | | 9.66 ± 1.78 | 3.58 ± 1.32 | 5.21 ± 0.72 | 1968 |
| *Periclistus brandtii* | | 1.13 ± 0.62 | 1.87 ± 0.42 | 4.89 ± 1.32 | 765 |
| *Orthopelma mediator* | D | 4.39 ± 0.77 | 2.77 ± 0.63 | 3.62 ± 0.83 | 1109 |
| *Glyphomerus stigma* | D,P | 0.98 ± 0.21 | 3.53 ± 0.63 | 3.61 ± 0.46 | 748 |
| *Torymus bedeguaris* | D | 1.75 ± 0.42 | 2.34 ± 0.32 | 1.51 ± 0.44 | 562 |
| *Pteromalus bedeguaris* | D | 1.49 ± 0.17 | 1.37 ± 0.37 | 0.89 ± 0.22 | 377 |
| *Caenaxis inflexa* | P | 0.07 ± 0.03 | 0.78 ± 0.30 | 2.60 ± 0.67 | 326 |
| *Eurytoma rosae* | P | 0.07 ± 0.03 | 0.51 ± 0.12 | 0.67 ± 0.16 | 130 |
| *Eupelmus urozonus* | D,P | 0.33 ± 0.17 | 0.45 ± 0.12 | 0.30 ± 0.09 | 105 |
| *Torymus rubi* | D | 0.03 ± 0.02 | 0.12 ± 0.07 | 0.19 ± 0.06 | 32 |
| *Torymus* spp. | | 0.11 ± 0.04 | 0.03 ± 0.02 | 0 | 16 |
| Indefinable | | 0.18 ± 0.07 | 0.10 ± 0.08 | 0.05 ± 0.03 | 37 |

At eight localities across Germany 388 galls were sampled on 135 shrubs (Table 3.1). In 90 galls no gall inhabitant developed. For further analysis we used the galls with one or more

developed individuals (N = 298 galls). In total 6,175 individuals emerged from the sampled galls (Table 3.1).

Of these 31.9% were *D. rosae*, 12.4% were the inquilin *P. brandtii* and 55.7% were parasitoids of *D. rosea* as well as *P. brandtii*. The most abundant parasitoids of *D. rosae* were *O. mediator* with 18.0%, *G. stigma* with 12.1% and *T. bedeguaris* with 9.1%. The parasitoids of the inquilin *P. brandtii* (*C. inflexa* and *E. rosae*) made up just 7.4% of all individuals. The remaining parasitoids *P. bedeguaris*, *E. urozonus*, *T. rubi* and *T.* spp. contributed 9.2%.

The gall volume was positively correlated with the number of gall chambers ($r^2$ = 0.54, $p<0.001$) and the number of inhabitants per gall ($r^2$ = 0.44, $p<0.001$). In the glm the gall volume depended on the sampling site and the interaction between inhabitants and site (Table 3.2). But it was independent of the rose species. The number of inhabitants per gall was also highly correlated with the number of gall chambers ($r^2$ = 0.72, $p<0.001$). And in the glm the number of inhabitants depended on the sampling site, but was independent of the rose species (Table 3.2).




Values <0 not shown

Fig. 3.4. Varinace partitioning of the parasitoid community within *Diplolepis rosae* galls sampled at eight sites (see Fig. 3.1) on three host plant species (*Rosa rubiginosa*, *R. corymbifera* and *R. canina*).

The rate of parasitism of *D. rosae* as well as *P. brandtii* varied between sampling sites and decreased with increasing gall volume (Table 3.2, Fig. 3.3). All two way interactions were significant, pointing to complex effects of geography and rose species on the communities associated with *D. rosae* galls.

The gall volume explained 16 % of the variation within the abundance of the parasitoids' community, the sampling site 1 % and the rose species 4 % (Fig. 3.4). All single joint fractions between two or three variables explained not more than 1% of variation. All in all the variables explained 22 %.

Table 3.2. Effects on the number of galls per shrub (N = 251 shrubs), the parasitism rate of *Diplolepis rosae* complex (N = 292 galls), the parasitism rate of the *Periclistus brandtii* complex (N = 204 galls), the gall volume (N = 386 galls) and the number of inhabitants (N = 388 galls), respectively. PC1 and PC2 are two principle components calculated in a PCA (see text). PC1 characterizes the size of a shrub and PC2 the habit of a shrub. Results are approached with generalised linear models (count data: Poisson distribution, log link function; proportion data: binomial distribution, Logit link function). DV = deviance, significance (* p<0.05, ** p<0.01, *** p<0.001) is highlighted in bold.

| | **Galls (shrub$^{-1}$)** | | ***Diplolepis*-complex** | | ***Periclistus*-complex** | | **Gall volume (GV)** | | **Inhabitants (I)** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DV | | DV | | DV | | DV | | DV | |
| PC1 | 1.78 | $F_{1,160} = 1.74$ | | | | | | | | |
| PC2 | 28.31 | **$F_{1,160} = 27.68$*** ** | | | | | | | | |
| Site (S) | 48.45 | **$F_{16,160} = 2.96$** ** | 345 | **$F_{7,256} = 9.11$*** ** | 171 | **$F_{7,182} = 4.26$*** ** | 55 | **$F_{7,262} = 6.10$*** ** | 7 | **$F_{7,274} = 3.56$** ** |
| GV | | | 479 | **$F_{1,256} = 88.73$*** ** | 63 | **$F_{1,182} = 10.96$** ** | | **$F_{1,262} = 162.16$*** ** | | |
| I | | | | | | | | $F_{2,14} = 0.01$ | | |
| Host (H) | 19.53 | $F_{2,32} = 0.57$ | 192 | $F_{2,14} = 2.53$ | 57 | $F_{2,14} = 0.71$ | 208 | | | |
| PC1xPC2 | 0.44 | $F_{1,160} = 0.43$ | | | | | 0 | | 80 | $F_{2,14} = 0.23$ |
| PC1xS | 53.81 | **$F_{16.160} = 3.29$*** ** | | | | | | | | |
| PC1xH | 2.01 | $F_{2,160} = 0.98$ | | | | | | | | |
| PC2xS | 43.07 | **$F_{16,160} = 2.63$** ** | | | | | | | | |
| PC2xH | 13.93 | **$F_{2,160} = 6.81$** ** | | | | | | | | |
| SxGV | | | 163 | **$F_{7,256} = 4.31$*** ** | 79 | $F_{7,182} = 1.96$ | | | | |
| GVxH | | | 4 | $F_{2,256} = 0.41$ | 9 | $F_{2,182} = 0.79$ | | | | |
| SxH | 71.52 | **$F_{32,160} = 2.19$** ** | 173 | **$F_{14,256} = 2.29$** ** | | | 19 | $F_{14,262} = 1.03$ | 446 | $F_{14,274} = 1.33$ |
| IxS | | | | | | | 18 | $F_{7,262} = 2.02$ | | |
| IxH | | | | | | | 14 | **$F_{2,262} = 5.41$** ** | | |
| Residuals | 104.78 | | 1324 | | 806 | | 302 | | 5454 | |

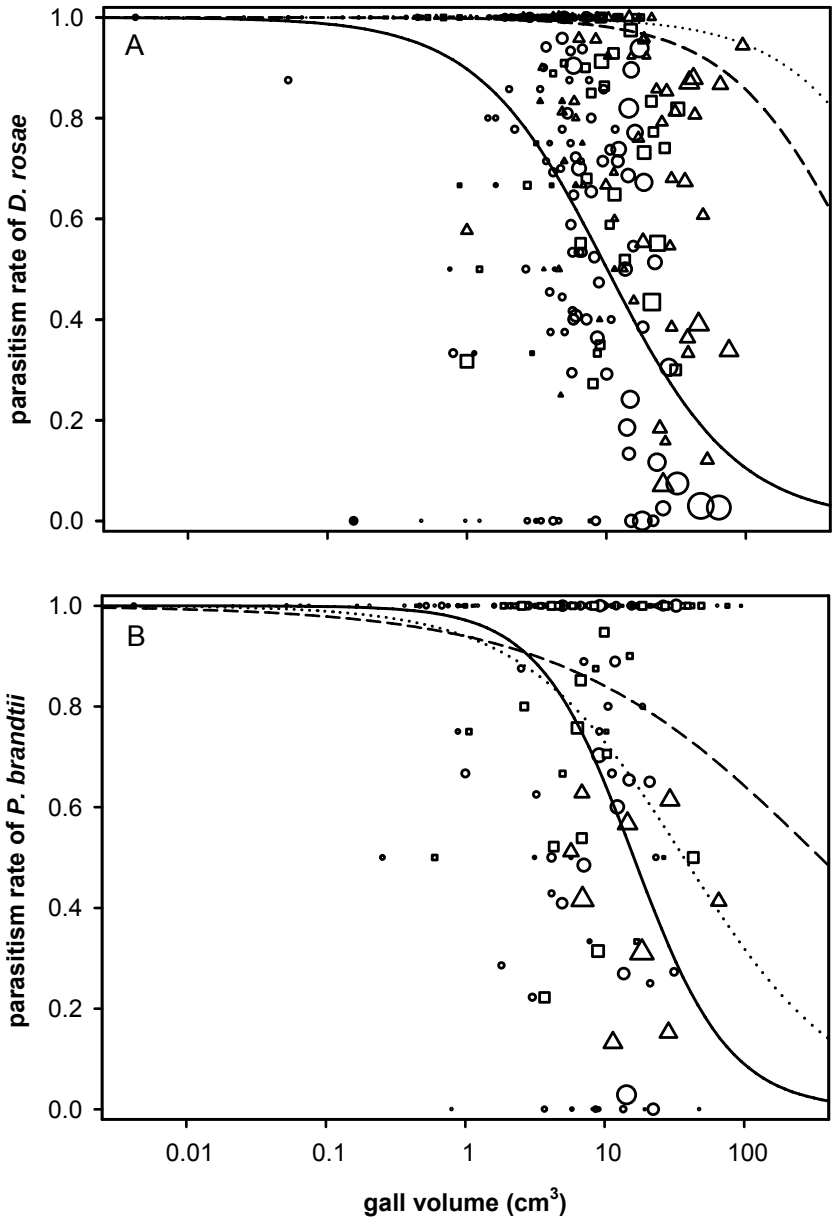



Fig. 3.3. Parasitism rate of **A**. *Diplolepis rosae* and **B**. *Periclistus brandtii* on three different host species *Rosa rubiginosa* (solid line), *R. corymbifera* (dashed line) and *R. canina* (dotted line). The gall volume was log transformed (lnGV). Symbols show galls from *R. rubiginosa* (circles), *R. corymbifera* (squares) and *R. canina* (triangles). The extent of the symbols are in proportion to the number of inhabitants per gall. The figure is based on a generalised linear model with binomial distribution, a mean site is chosen.

## 3.4 Discussion

The one inquiline and all parasitoid species expected within Europe were found with frequencies comparable to other parts of Europe. A survival rate of 31% for *D. rosae* was much higher than reported by Schröder (1967) for southwest Germany (9.7%). But it is comparable with populations in Spain showing the highest survival rate (35%) within Europe (Schröder 1967, Randolph 2005).

Our analyses can be summarised in three main results. First, the densities and abundances of the *D. rosae* gall community are independent of the host species. Second, abundances varied highly with the geographical location. And third, there is a complex interaction between the host plant species and the geographical location, which suggest that environmental variations among sites influences the habits of individual shrubs and therefore alter the host choice of *D. rosae* and the associated community of parasitoids.

*Gall characteristics do not depend on the rose species*

Gall characteristics such as the shape and toughness are plant derived structures, but often regulated by insect genes whereas the gall diameter for example can differ between host plant species, thereby affecting multitrophic interactions (Weis & Abrahamson 1985, Price & Clancy 1986b). Price and Clancy (1986b) showed that genetic variation among clones of the willow *Salix lasiolepis* affected gall size. In turn the galls' sizes and densities determine the success as well as composition of parasitoid communities (Weis 1983, Brandl & Vidal 1987; Schlumprecht 1989).

Larger gall size has also been correlated in other gall forming insect species with increased larval survival and decreased levels of parasitism (Price & Clancy 1986b, Stiling & Rossi 1997). Obviously the length of the parasitoid`s ovipositor limits oviposition (Stille 1984, Price & Clancy 1986a, Brandl & Vidal 1987, Stiling & Rossi 1997). The variation in the composition of the parasitoid`s community explained by the gall volume points to different parasitoid preferences. The most common parasitoid, *O. mediator*, a large and host specific endoparasitoid, enters the community early, even at the same time as the gall-maker itself (Schröder 1967, Stille 1984, Randolph 2005). Since *O. mediator* appears so early it probably deposits the eggs even before the gall has started to develop. If this is the case practically all host larvae within the gall could be reached, regardless of the galls volume. Both other common parasitoids *G. stigma* and *T. bedeguaris* are ectoparasitoids and emerge considerably later around middle or end of June (Schröder 1967, Randolph 2005) and should be more dependent on gall size.

*No clear impact of the host plant species*

The rose species is only indirectly important for the choice of gall wasps, depending on the amount of developing shoots available, although the three rose species differ in several characters: *R. canina* is a glabrous rose, *R. corymbifera* has hairs on rhachis and abaxial leaf surface and *R. rubiginosa* has glandular trichomes on the lower leaf surface. The three rose species also differ in plant architecture and phenology (Timmermann 1998; Wissemann et al. 2006). Developing leaves or tips of shoots are used by the gall wasps for transformation into galls (Schröder 1967). Most of the galls were found on the glandular rose *R. rubiginosa*, which is slightly taller and shows a denser habit with more developing shoots. Similar results were found for galling insects on species of the genus *Baccharis*. Here, the most important architectural features governing the number of galls, was the number of fourth-level shoots (Espirito-Santo et al. 2007).

Infestation of developing shoots could lead to a decrease of annual growth and may reduce the number of flowers and fruit. However, this negative effect on host plant growth only occures at high cynipid densities (Crawley & Long 1995, Kato & Hijii 1997). Although Schröder (1967) reported high densities (up to 60 galls per single shrub) and hence fitness consequences, the densities of *D. rosae* are normally low. A mean infestation rate of 0.4 galls per shrub was observed. Significant effects of gall wasps on the population dynamics of the hosts are rare (Stone et al. 2002). Therefore there is no need for the host species to co-evolve and adapt characters defending themselves against the gall wasps, e.g. attracting parasitoid species or suppress gall formation. Accordingly we found no differences in the parasitism rate of parasitoids and the survival rate of the gall wasp between different host species. In our case the glandular trichomes of *R. rubiginosa*, which are rich in secondary metabolites belonging to the sesquiterpene group (Klinge, unpublished data) seem not to affect the parasitoid community.

Even today there is ongoing and permanent gene flow by hybridisations between rose species of this section. The gene flow between roses and the reticulate speciation process may act as a hybrid bridge. This hypothesis (Floate & Whitham 1993) interprets hybrids as connections between species on which herbivores can change from one host to another. So far no specialisation of rose specific species between members of the section *Caninae* were detected neither in rust fungi of the genus *Phragmidium* (Ritz et al. 2005a) nor in insects, especially the rose hip fly *Rhagoletis alternata* (Vaupel et al. 2007).

*The gall-parasitoid community differs on the rose plant species at different geographical locations*

The plant-gall-parasitoid interaction was not stable between geographical locations. Similar changes in host-by-environment interactions are found in the arthropod community of the evening primerose *Oenothera biennis* (Johnson & Agrawal 2005). Tscharntke et al. (2001) detected even differences in the composition of parasitoids of grass-feeding chalcid wasps as well as differences in patterns of interactions between Britain and Germany.

Maybe the three rose species have somewhat different habitat requirements. One rose species had more developing shoots at one location than the others and would therefore be more frequently infested. However, even on the same localities we found highly differing gall numbers. Some authors have hypothesized, that dog roses are able to suppress gall formation (e.g. Schröder 1967). The mechanism remains unknown, but roses in optimal physiological conditions should be more successful in protecting themselves. Oviposition would occur on vigorous and on weak plants, but gall formation should only be successful on the weak plants (Schröder 1967) hinting at internal plant defensive abilities. Therefore Schröder (1967) argued that gall density should increase with environmental stress (e.g. drought). This is in contradiction to our results. One would expect that vigorous plants are in better physiological condition and therefore developing more shoots. According to our results these shrubs should have a higher density of galls and not the other way around. Consistently, in southern Sweden, Stille (1984) observed more and larger galls on young shrubs and on shrubs along roadsides which were frequently damaged and produced frequent and large shoots.

*Conclusion*

The aim of our study was to analyse how specific differences of closely related host plants translate into higher trophic levels of insect communities. The host plant species had no direct influence, neither on the gall densities, nor on the gall volume, nor the parasitism rates, nor the communities associated with *D. rosae* galls. However, interactions between host plant species and herbivore communities changed depending on the geographical location. Therefore we conclude that the reticulate and ongoing hybridisation of dog rose species prevents clear host preferences of the herbivores and their dependent community or even specialisation of exploiters. Nevertheless, the host species in combination with the locality plays an important role and shows a complex influence on exploiter abundances. Therefore, the plant-gall-parasitoid interaction is mainly influenced by local environmental conditions which could alter host choice and exploiter densities.

## ACKNOWLEDGEMENTS

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# 4. No host-associated differentiation in the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae) on three dog rose species

Annette KOHNEN, Volker WISSEMANN and Roland BRANDL

ABSTRACT

Differences in secondary compound quality and quantity and leaf structure of host plants may influence the host choice of exploiters, which could lead to host-associated genetic differentiation of the exploiters. Here we investigated whether the rose gall wasp *Diplolepis rosae* L. (Hymenoptera: Cynipidae) genetically adapted to three closely related host plants (*Rosa canina* L.*, Rosa corymbifera* Borkh. and *Rosa rubiginosa* L.), which recently radiated and subsequently expanded their range. Because the plant tissue is manipulated by the gall wasp a close evolutionary relationship between the wasp and its host is expected. The parthenogenetic reproduction of *D. rosae* induced by strains of the bacterial genus *Wolbachia* could influence both the host-associated genetic structure and geographic patterns. We found that almost 100% of the *D. rosae* individuals were infected with *Wolbachia* sp. Using 106 polymorphic AFLP markers we found no genetic differentiation among the wasps from different host plants and from different geographic locations. The ongoing hybridisation within the genus *Rosa* may act as a "hybrid bridge", preventing adaptation of *D. rosae* to one specific host plant. The lack of geographical structuring could be explained by large population sizes and the good dispersal ability of *D. rosae* combined with low genetic variation owing to *Wolbachia* infection.

## 4.1 Introduction

Plant traits, including secondary compounds, plant architecture, and nutritional value, vary even among closely related species (e.g. Orians 2000, Pichersky & Gang 2000, Malmstrom et al. 2000). This variation in plant traits can influence the abundance of herbivorous insects as well as the structure and dynamics of associated herbivore communities (e.g. Cattell & Stiling 2004, Bailey et al. 2006). Even plant traits encoded by few genes may have important effects on the community of plant exploiters, as suggested by the concept of the "extended phenotype" (Whitham et al. 2003). Differences in morphological traits could lead to host preferences of herbivorous insects and, as a consequence, to adaptations to certain host species. Adaptations may lead to genetically differentiated populations of exploiters living in sympatry, i.e. to host races (Dres & Mallet 2002). The formation of host races is therefore a first step towards sympatric speciation. Host races have been found within a variety of species, e.g. fruit flies (Bush 1969, Diegisser et al. 2004), thrips (Brunner et al. 2004) and lepidopterans (Sperling et al. 1999). One of the most well-known examples is the apple fruit fly *Rhagoletis pomonella* which underwent a host shift from fruits of *Crataegus* sp. to those of *Malus* sp. The fruits of the two hosts differ in their phenology, which selects for adaptive differences of the two fruit fly populations (Feder et al. 1988). Host-associated genetic differentiation has been found in even more taxa, not only in specialised herbivorous insects (e.g. Stone et al. 2009), but also in polyphagous grasshopper populations (Sword et al. 2005). In most of these cases, the host plants used by a single insect species are distantly related species (but see Eber et al. 1999).

Wild roses of the section *Caninae* (DC) Ser. are thought to have originated by allopolyploid hybridisation events and subsequent spread throughout Europe after the last glacial period (Wissemann 2002, Ritz et al. 2005b). Although closely related, the rose species of this section differ in several characters, e.g. in plant architecture (Wissemann et al. 2006) and phenology (Timmermann 1998), which may influence the abundance and interactions with associated exploiters. Three widely distributed and abundant species that often occur in the same habitats in central Europe are *Rosa canina* L.*, Rosa corymbifera* Borkh., and *Rosa rubiginosa* L. They differ in several characters, in particular in density and type of trichomes on the lower leaf surface: *R. canina* is a glabrous rose, *R. corymbifera* has hairs on the rhachis and abaxial leaf surface, and *R. rubiginosa* has glandular trichomes on the lower leaf surface. Trichomes are believed to influence host choice of herbivores as well as of other invertebrates (e.g. Zvereva et al. 1998, Ranger & Hower 2002).

Plant galls develop as a result of interactions between the inducing insect and the host, wherein the insect gains control and redirects the growth and physiology of attacked organs

to the insect's advantage (Shorthouse et al. 2005a). Because of the intimate relationship between plants and gall-inducing insects, a close co-evolution is expected. With 1300 described species, the gall wasps (Cynipidae) are the second largest family of gall-inducing insects (Liljeblad & Ronquist 1998). Gall wasps are small insects that form complex and well-organised galls on various plant species and are found on all plant organs (flowers, leafs, buds, stems, twigs and roots). Gall characteristics, such as shape and toughness, are plant-derived structures but are regulated by insect genes, whereas the gall diameter, for example, could be regulated by the plant genotype (in *Salix lasiolepis*; Price & Clancy 1986a). Owing to the physiological manipulation of the plant, gall wasps should be closely adapted to specific host plants (Crawley & Long 1995, Kato & Hijii 1997). Differences in secondary compounds and leaf structure of host species may furthermore influence the host choice of gall wasps.

The univoltine, cynipid gall wasp *Diplolepis rosae* L. (Hym.) makes conspicuous and multi-chambered galls, which have been found on *Rosa* species from several sections (Schröder 1967). Host-associated differentiation in the genetic structure of this gall wasp is expected because of its parthenogenetic reproduction, which is induced by bacteria of the genus *Wolbachia* (Schilthuizen & Stouthamer 1998). These intracellular bacteria are common and have been detected in a wide range of arthropod species (Werren 1997, Werren et al. 2008). These micro-organisms are transmitted by the cytoplasm of egg cells and alter reproduction of their hosts in several ways, including cytoplasmic incompatibility, male killing, feminisation, and induction of parthenogenetic development (Stouthamer et al. 1999). Because the insect reproduction is modified, *Wolbachia* infection also has a long-term impact on the evolution of host taxa and their genetic structure. Parthenogenesis-inducing *Wolbachia* strains could reduce gene flow between populations (Werren et al. 2008) and genetic variability (Plantard et al. 1998) up to reduction of populations into clones without genetic exchange, which should accelerate differentiation processes in populations. Because of this *Wolbachia*-induced parthenogenesis, we expect delimited gene flow between populations of *D. rosae* on different host species. Here, we report our investigation at four sites in Germany of the host-associated genetic differentiation of *D. rosae* on three closely related host plant taxa, *R. canina*, *R. corymbifera*, and *R. rubiginosa*, which recently underwent a flush of radiation. The genetic structure of *D. rosae* was assessed using amplified fragment length polymorphism (AFLP). Additionally, we investigated the geographical differentiation and the *Wolbachia* sp. infection rate.

## 4.2 Material and Methods

*Sampling*

We sampled galls of *D. rosae* at four locations in Germany during October 2006 and 2007 (Table 4.1) on three rose species (*R. canina* L., *R. corymbifera* Borkh., and *R. rubiginosa* L.). The sampling site in Göttingen is within the botanical garden, where different rose species and their hybrids were planted for an experiment from a previous study of the second author (V. Wissemann). This collection is now housed at the Justus Liebig University, Giessen. At all sites, the three host plant species occurred within the same habitat.

All sampled galls were kept individually outside from October to July in plastic pots closed covered with gauze. All inhabitants were allowed to emerge until July, galls were then dissected. Inhabitants were stored in 90% alcohol at 4 °C.

Table 4.1. Geographical locations of sampling sites in Germany and sample size of *Diplolepis rosae* galls on different rose species.

| Site | Latitude | Longitude | Sample size | Host plant (*Rosa*) |
|---|---|---|---|---|
| I. Jena | 50.94472 | 11.57792 | 14 | *R. canina* |
| | | | 9 | *R. corymbifera* |
| | | | 15 | *R. rubiginosa* |
| II. Kahla | 50.80111 | 11.60510 | 14 | *R. canina* |
| | | | 12 | *R. corymbifera* |
| | | | 14 | *R. rubiginosa* |
| III. Würzburg | 49.8000 | 9.9333 | 21 | *R. canina* |
| | | | 14 | *R. corymbifera* |
| | | | 21 | *R. rubiginosa* |
| IV. Göttingen | 51. 5333 | 9.84375 | 1 | *R. canina* |
| | | | 1 | *R. corymbifera* |
| | | | 13 | *R. rubiginosa* |
| | | Sum | 149 | |

*DNA Extraction*

Because *D. rosae* reproduces parthenogenetically, individuals within one gall are expected to be identical. Therefore, only one individual per gall was used for genetic analyses. AFLP protocols are known to be sensitive to contaminations (Savelkoul et al. 1999), especially to DNA of micro-organisms abundant in the digestive tract of insects. To avoid such contaminations, total DNA was extracted only from the legs. The legs were ground in 1.5 ml microfuge tubes using steril plastic pestles. DNA was extracted using spin columns (DNeasy tissue kit, Qiagen, Hilden, Germany) following the manufacturer's protocol for animal tissue.

*AFLP methods*

The genetic diversity of *D. rosae* was analysed by AFLP according to Vos et al. (1995). For each individual, 9 µl of DNA (50–100 ng) was restricted with 1 unit *Eco*RI and 1 unit *Mse*I (2 h at 37 °C, followed by 20 min at 70 °C). The digested DNA in the samples was ligated with T4 DNA ligase to adaptors (AFLP Core Reagent Kit, Invitrogen, Karlsruhe, Germany) at 20 °C for 2.5 h. The mixture was diluted (1:10) and used as a template (2 µl) in the pre-selective PCR, using *Mse*+C and *Eco*+A primers in the AFLP Pre-amplification Primer Mix I (Invitrogen) and *Taq* DNA polymerase (New England Biolabs, Frankfurt, Germany) in a reaction volume of 25 µl under the following conditions: an initial denaturation cycle at 95 °C for 5 min, followed by 20 cycles at 94 °C for 30 s, annealing at 56 °C for 1 min and extension at 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min.

The selective PCR reactions (11 µl) contained 1 µl of pre-selective DNA (diluted 1:40), 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 80 µM dNTP, 2 µM of each primer with three selective base pairs at the 3'-end (Table 4.2) and 1 unit of *Taq* DNA polymerase (New England Biolabs). After testing 45 different primer combinations, five combinations were chosen for final analysis (Table 4.2). The *Eco*RI-primers were fluorescently labelled with IRDye-700 or IRDye-800 (Metabion, Martinsried, Germany). The reactions were carried out in a touchdown reaction with the following protocol: initial denaturation cycle at 94 °C for 5 min, one cycle of denaturation at 94 °C for 30 s, annealing for 30 s at 65 °C and then extension at 72 °C for 1 min, followed by 12 cycles in which the annealing temperature was lowered to 56.6 °C in 0.7 °C steps. This was followed by 23 cycles at an annealing temperature of 56 °C and a final extension step at 72 °C for 5 min. For all amplifications, a thermocycler (Eppendorf Mastercycler, Hamburg, Germany) was used.

The PCR products were diluted (1:5) and visualised on 8% Long Ranger polyacrylamide gels (Biozym, Oldendorf, Germany) running on a LI-COR DNA Analyser (LI-COR, Bad Homburg, Germany) for 4.5 h (40 W, 1500 V). Individuals were arranged on gels by gall numbers, which do not correspond with the sampling order (sampling sites or rose species). Data were processed using SagaMX software (LI-COR). Samples were manually checked for correct alignment of the size standard and corrected if necessary. The presence (1) or absence (0) of 100-500 bp fragments was scored. Samples with weak or noisy signals were noted and re-analysed. All scoring was done blind with regard to the population's origin and controlled according to the criteria of Bonin et al. (2004). The error variability between gels was calculated to be around 2% using AMOVA (Table 4.3).

Table 4.2. Primer, primer combinations and dye labelling used in AFLP analyses.

| Primer | Sequence 5´→ 3´ | Labelling (IRDye) | Number of loci |
|---|---|---|---|
| Pre-selective | | | |
| *Mse*-C | GATGAGTCCTGAGTAAC | | |
| *Eco*-A | GACTGCGTACCAATTCA | | |
| Selective | | | |
| *Mse*-CAT | GATGAGTCCTGAGTAACAT | | |
| *Mse*-CAC | GATGAGTCCTGAGTAACAC | | |
| *Mse*-CTT | GATGAGTCCTGAGTAACTT | | |
| *Eco*-ACG | GACTGCGTACCAATTCACG | 700 | |
| *Eco*-ACC | GACTGCGTACCAATTCACC | 700 | |
| *Eco*-AGC | GACTGCGTACCAATTCAGC | 800 | |
| Primer combinations | | | |
| S1-700 | *Mse*-CAT / *Eco*-ACG | 700 | 15 |
| S2-700 | *Mse*-CAT / *Eco*-ACC | 700 | 26 |
| S2-800 | *Mse*-CAC / *Eco*-AGC | 800 | 29 |
| S3-700 | *Mse*-CTT / *Eco*-ACG | 700 | 18 |
| S3-800 | *Mse*-CTT / *Eco*-AGC | 800 | 18 |

*Detection of Wolbachia sp*.

The presence or absence of *Wolbachia* sp. was tested by PCR with specific primer pairs amplifying ca. 600 bp of the *wsp* gene: forward *wsp* 81F (5´-TGG TCC AAT AAG TGA TGA AGA AAC-3´), reverse *wsp* 691R (5´- AAA AAT TAA ACG CTA CTC CA-3´; Braig et al. 1998). Whether the absence of a PCR product was caused either by the absence of *Wolbachia* sp. cells or by a failure in the reaction was checked with a control primer pair of 180 bp: forward Dr06-F (5´- CTC ATC TCT TCT TCT TAT CTC AG-3´) and reverse Dr06-R (5´-CCC AGG AGA GCA GAG G-3´ (Plantard et al. 1998). With all PCR reactions, a positive (known infected individual) and a negative (water) control was run. DNA was amplified using an initial denaturation step at 94 °C for 3 min, then 35 cycles with denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 1 min. These steps were followed by a 5 min extension at 72 °C.

The PCR reactions were carried out in 2-5 µl of template DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 80 *µ*M dNTP, 10 *µ*M of each primer and 1 unit of *Taq* DNA polymerase (New England Biolabs) in a 20 µl total. PCR products were visualised on an agarose gel (2%) and stained with ethidium bromide (2 $mgl^{-1}$, 20 min).

As a further control, the *wsp* fragment of six individuals was sequenced. PCR products were purified using a Qiagen MinElute PCR purification kit and sequenced directly by Sequencing Laboratories Göttingen GmbH, Germany.

*Statistics*

To evaluate the error associated with scoring of bands, we analysed the molecular variance (AMOVA; Excoffier et al. 1992) to estimate the variability between individuals scored on different gels using Arlequin version 3.11 (Excoffier et al. 2005).

Subsequently, the genetic structure of the 149 *D. rosae* individuals was assessed using three approaches: 1) geographical structure between sampling sites, 2) spatial structures between and within sampling sites, and 3) differences between host species. We calculated pair-wise $F_{ST}$ values (Weir & Cockerham 1984) between sampling sites with Arlequin using Bonferroni corrections to adjust for multiple testing. An AMOVA was conducted to calculate variance components of genetic variation and their significance levels for variation between individuals of *D. rosae* within and among sample sites. Additionally, this method was used to partition the variance between host species within sampling sites and among sampling sites. Levels of significance were determined with 10,100 random permutations.

To test for spatial genetic structure assignment tests were performed using Structure 2.2 (Pritchard et al. 2000). With this program, we determined the most likely number of genetic clusters (K) that best fits the data. Likelihood estimates were calculated ten times for each level of K (1-7). Using the admixture ancestry model with correlated allele frequencies, our analysis implemented a burn-in period of 10,000 iterations and a Markov chain of 10,000 iterations for data collection. The output from these runs was used to evaluate ΔK using the formula of Evanno et al. (2005), which is assumed to show a peak at the true value of K.

Spatial genetic structure was evaluated by autocorrelation analysis in GenAlEx 6.2 (Peakall & Smouse 2006). The autocorrelation coefficient (r) is a true correlation coefficient ranging from –1 to 1 and measures the genetic similarity between individuals (Smouse & Peakall 1999). Error values were determined by bootstrap re-sampling (1,000 replicates) and 95% confidence limits for r about the null hypothesis (no genetic structure) were estimated by re-sampling (999 permutations). Distance classes were chosen according to the distribution of study sites and sample size.

## 4.3 Results

Using five primer combinations, we unambiguously scored only 106 polymorphic markers (ca. 46%). No two individuals were identical we found 149 different haplotypes. Pair-wise differences ranged from 2 to 50, and genetic distances accordingly ranged from 0.013 to 0.331. We checked 138 individuals for infection with *Wolbachia* sp. of which 137 (>99%) were infected. In a Blast search, the sequenced *wsp* fragment from six sequenced individuals (all identical, 550 bp) showed 98% identity to that of a *Wolbachia* sp. endosymbiont of *Tetranychus urticae*.

Two per cent of the variation between individuals in the AFLP data was explained by arrangements of individuals on different gels (Table 4.3). Individuals were not randomly distributed among the gels; therefore, this effect is a lower limit because individuals from the same sampling site were often scored on the same gel (see above).

Table 4.3. Results of AMOVA

| Source of variation | d.f. | Sum of squares | Variance | % total | Φ statistics | P |
|---|---|---|---|---|---|---|
| *Gels* | | | | | | |
| Among gels | 3 | 61.51 | 0.25 | 2.11 | | |
| Within gels | 145 | 1649.99 | 11.38 | 97.89 | | |
| Total | 148 | 1711.50 | 11.62 | | $\Phi_{ST}$ = 0.021 | <0.0001 |
| *Sampling sites* | | | | | | |
| Among sites | 3 | 53.88 | 0.18 | 1.54 | | |
| Within sites | 145 | 1657.61 | 11.43 | 98.46 | | |
| Total | 148 | 1711.50 | 11.61 | | $\Phi_{ST}$ = 0.015 | <0.001 |
| *Sampling site – rose species* | | | | | | |
| Among sites | 3 | 53.59 | 0.07 | 0.64 | $\Phi_{CT}$ = 0.006 | 0.25614 |
| Among species, within sites | 6 | 92.39 | 0.29 | 2.56 | $\Phi_{SC}$ = 0.026 | <0.001 |
| Within species | 137 | 1519.14 | 11.09 | 96.80 | | |
| Total | 146 | 1665.12 | 11.45 | | $\Phi_{ST}$ = 0.032 | <0.0001 |

Only a minor geographical structure between the sites was found: Pair-wise $F_{ST}$ values between sample sites ranged from 0.0058 to 0.043 (Table 4.4). After Bonferroni correction, only one comparison was significant. The sampling site explained just 1.5% in the AMOVA, less than our error rate (Table 4.3). The overall $\Phi_{ST}$ value (0.015) was nevertheless significant. In a nested AMOVA design, the rose species explained 2.5% ($\Phi_{SC}$ = 0.026, $p < 0.001$) of variance, and the sampling site (0.64%) showed no significant contribution to total variance ($\Phi_{CT}$ = 0.006, p = 0.25614, Table 4.3).

Table 4.4. Pair-wise comparisons of $F_{ST}$ values between sampling sites and their significance. Below the diagonal, significant $F_{ST}$ values (< 0.05) are highlighted in bold. Above the diagonal, significant p values after Bonferroni correction (< 0.008) are highlighted.

| | $F_{ST}$ values | | | |
|---|---|---|---|---|
| Study site | I | II | III | IV |
| I. Jena | --- | 0.036 | 0.117 | 0.027 |
| II. Kahla | **0.012** | --- | 0.018 | **0.001** |
| III. Würzburg | 0.006 | **0.017** | --- | 0.036 |
| IV. Göttingen | **0.016** | **0.043** | **0.012** | --- |

The assignment test suggested most likely two genetic groups in our data set because the likelihood values increased between one and two assumed groups (Ln = -6715, Ln = -6082, respectively), with more groups likelihood values saturated. Accordingly, ΔK showed a peak at two groups (ΔK = 46.8) and decreased to values below four with more assumed groups. However, these two clusters did not correspond with sampling sites, geographical regions, or host species origin.

Autocorrelation analysis indicated a spatial structure in which genotypes are more similar than expected from chance at distances less than 300 m for all data combined and less than 50 m within one representable study site (Fig. 4.1).

## 4.4 Discussion

In our study of the host-associated differentiation of *D. rosae* on three species of the genus *Rosa*, three main results were observed. First, we found low but significant genetic differentiation of *D. rosae* individuals on the three rose species. However, the differences were within the error rate of the method. Therefore, we do not interpret this as an indication of host-associated differentiation or even of the formation of host races. Second, we found low geographical structure between sampling sites. Again, the differences were within the error rate. Third, *D. rosae* showed an infection rate with *Wolbachia* sp. of almost 100%.

One explanation for the low genetic differentiation of *D. rosae* individuals on the three host plants is a young association with the host species. The radiation and speciation process resulting in the rose species of the section *Caninae* occurred during the last ice age (Wissemann 2002, Ritz et al. 2005b) at least 10,000 years ago. However, the fruit fly *Rhagoletis pomonella* formed host races with clear genetic differentiation within less than 150 years (Bush 1969). Therefore, this argument is not entirely convincing.

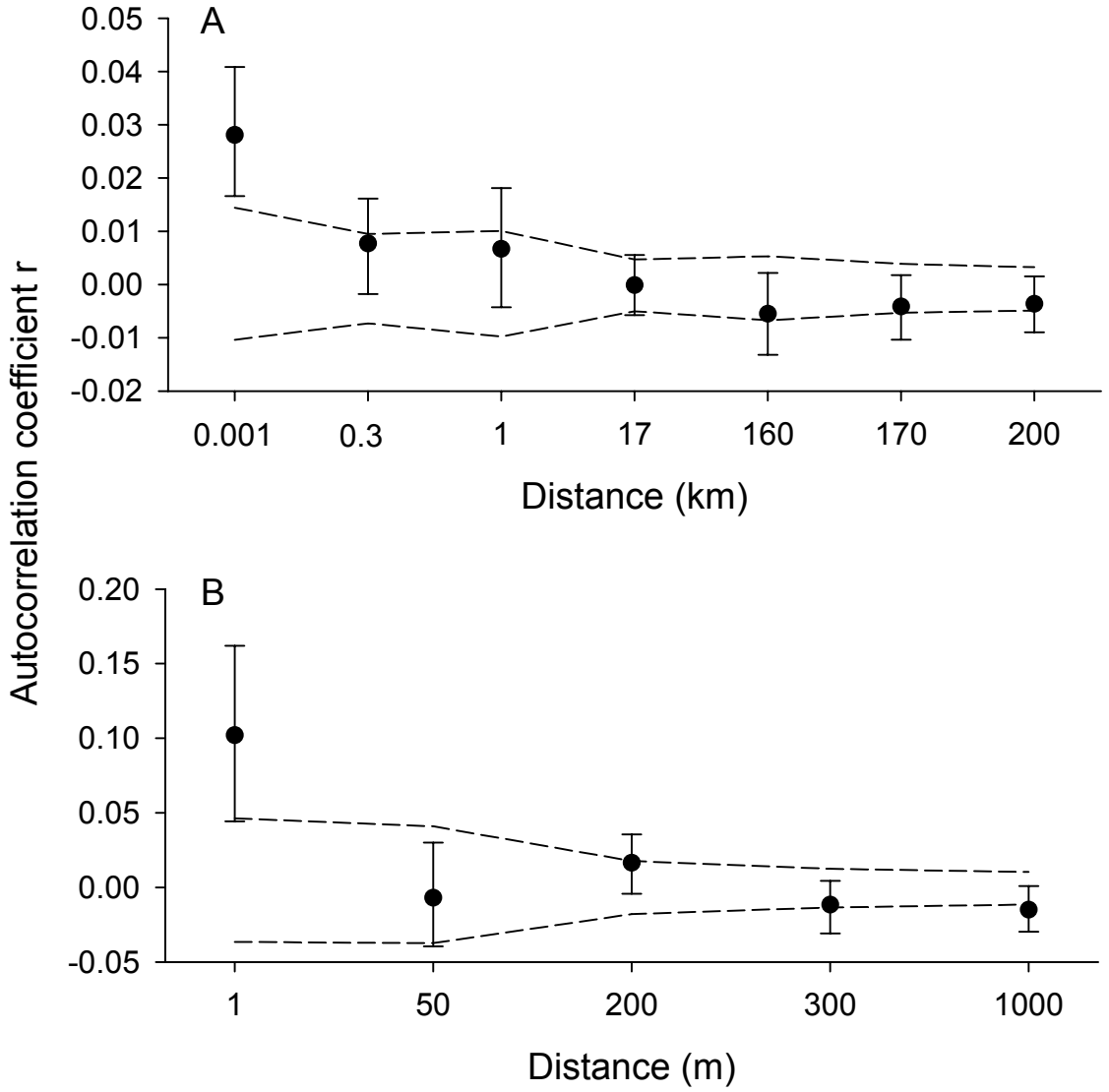


Figure 4.1. Spatial genetic structure of Diplolepis rosae individuals analysed with AFLP data **A**. over the whole study area and **B**. only within one study area (II. Kahla). Autocorrelation coefficient r (± 95% confidence intervals derived from 1,000 bootstrap replicates) indicates a spatial structure in which genotypes are more similar than expected from random distribution if values are >0 and less similar if values are <0. Dashed lines show 95% confidence limits of expected coefficients (999 random permutations).

A more compelling explanation might be the ongoing hybridisation between rose species of the section *Caninae* and, therefore, a continual gene flow between rose species. These hybrids have all intermediate forms of species characters. The gene flow between roses and the reticulate speciation process may create a “hybrid bridge”. This hypothesis (Floate & Whitham 1993) interprets hybrids as connections between parental species closing morphological, genetic or spatial gaps, on which exploiters can change from one host to another in gradual steps. Underlying this hypothesis is the assumption that hybrids may vary in the qualitatively or quantitatively content of secondary compounds. For example hybrids may synthesise all or only some of the secondary compounds synthesised by their parents. For specialised exploiters, it should be easier to switch between host species after adaptation to an intermediate hybride of both parental species. To date no specialisation of rose-specific exploiters, including rust fungi of the genus *Phragmidium* (Ritz et al. 2005a) and insects (Vaupel et al. 2007) on different species of the section *Caninae* have been found.

To explain the low genetic differentiation between sampling sites, we propose that the gall wasp has recently and rapidly expanded its range. Such range expansions caused by bottlenecks due to colonisation or host-shift events could result in low levels of genetic variation (Harrison 1991). A shift from another host species to the genus *Rosa* seems unlikely because all gall wasps of the genus *Diplolepis* are specialists on members of the genus *Rosa*, which suggest a long co-evolution. As a specialist the distribution of the gall wasp depends on the distribution of its host. Roses and their associated exploiters expanded their range to central and northern Europe after the last glaciation (Dingler 1907). The distribution of the roses provided a platform for the gall wasps to re-colonise Europe. Today the distribution and density of roses is highly influenced by humans. Many species and cultivars are planted in parks, gardens and along waysides throughout central Europe. This may trigger the distribution of gall wasps and leads, in combination with high population sizes and probably good dispersal ability, to low genetic differentiation. However, on small scales (up to 50 m), we found a clear spatial structure; *D. rosae* individuals were more closely related than expected by chance. Every newly emerged female carries a high number of eggs (500-1000) in her ovaries (Schröder 1967, Stille & Dävring 1980). Females begin ovipositing after emergence, and most females continue ovipositing in the field for two to three weeks (Schröder 1967). Assuming up to 30 eggs per gall (Schröder 1967), one female can induce at least 15 galls, which she probably disperses among several buds. Considering the short life time of the female wasp, she would probably search for a rose stand with numerous possible egg-laying sites, thereby distributing a considerable number of her galls in one location.

Another plausible explanation for the low genetic differentiation is that the genetic structure is shaped by infection with *Wolbachia* sp. and subsequent parthenogenetic reproduction. An

infection not only leads to reproductive modifications, but also has a long term impact on the evolution of host taxa and their genetic structure. For example, infection with *Wolbachia* sp. reduces gene flow between populations (Werren et al. 2008) and genetic variability (Plantard et al. 1998). During the initial phase of *Wolbachia* infection, selective sweeps may reduce diversity of other cytoplasmically inherited markers, especially mtDNA, thereby producing a genetic signal similar to that produced by a population bottleneck with subsequent expansion (Hurst & Jiggins 2005). Selective sweeps not only reduce haplotype diversity but also lead to deviations from predictions based on neutrality (Johnstone & Hurst 1996, Rokas et al. 2001). Nuclear markers are expected to show reduced variability in concordance with mtDNA only if *Wolbachia* induces parthenogenesis in its host species (Rokas et al. 2001). However, *Wolbachia* infection does not necessarily lead to lower levels of genetic variation. Pannebakker *et al*. (2004) found similar genetic diversity in infected and uninfected populations of *Leptopilina clavipes* a parasitoid of *Drosophila*. They conclude that multiple clones coexist within populations derived by several infection events owing to horizontal transmission between parasitoids and host.

In contrast to the results of Pannebakker *et al.* (2004) Schilthuizen and Stouthammer (1998) excluded horizontal transmission between *D. rosae* and its parasitoids because most of the parasitoid wasps were infected with *Wolbachia* strains only distantly related with the *Wolbachia* strain of *D. rosae*. One hint for a high *Wolbachia* infection in *D. rosae* is that males are rare (McCallan 1940, Askew 1960, Schröder 1967, Stille 1984). Therefore, our finding of an almost 100% infection rate at our study sites, which led to parthenogenesis as the exclusive reproduction strategy, was not surprising. Thus, no genetic exchange through reproduction and recombination could occur between individuals. The low genetic differentiation between *D. rosae* populations would therefore support the assumption, that *D. rosae* is able to disperse well.

Nevertheless, we found no clear structuring of the *D. rosae* populations, neither between host plant species nor between geographical locations. The lack of *D. rosae* population structure between the host plant species could be explained by the ongoing hybridisation of the plant and the accompanying limited phenological boundaries between the host plants. These host plant hybrids may prevent the formation of host-associated differences of the wasps on the host plant. The lack of *D. rosae* population structure between geographical locations could be explained by the wasp recently expanding its range, supported by the distribution of the host plants and good dispersal ability of *D. rosae* or a selective sweep of *Wolbachia* sp. infection.

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# 5. Comparing geographical structures of one cynipid gall wasp with two specialised parasitoids in Europe

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## ABSTRACT

Environmental conditions and ecological traits influencing current phylogeographical structures should be similar for interacting species pairs like parasitoids and their hosts because they are often highly specialised and they share the same habitat. Therefore, similar phylogeographic population structures for host-parasitoid-pairs are expected. Here, we compare the geographical structures of the cynipid gall wasp *Diplolepis rosae* L. (Hym., Cynipidae) and two of its most common parasitoid species *Orthopelma mediator* Thunbr. (Hym., Ichneumonidae) and *Glyphomerus stigma* Fabr. (Hym., Thorymidae). We analysed insect individuals from Europe with two genes (COI and ITS 2). An additional factor influencing current geographical structures might be the infection with *Wolbachia* bacteria which alter the reproduction strategy, and thereby the dispersal ability, of its host. The infection rate with *Wolbachia* bacteria demonstrated quite different patterns: *D. rosae* 86%, *O. mediator* 32% and *G. stigma* was not infected. Contrary to our expectations, the geographical structure of the three species was incongruent. The gall wasp had the lowest genetic diversity with one major central clade, *O. mediator* showed a classical European distribution with one eastern and one western clade, whereas *G. stigma* had the highest diversity but no geographical structuring. Two main reasons are plausible, first the free living stages as adults with different possibilities to disperse and second the ability to switch between host species, if the primary host is not available.

## 5.1 Introduction

One key question in understanding the geographical structuring of species is the impact of interactions and dependencies between species. This includes the extent to which interacting species like parasitoid and host populations are structured on similar spatial scales. This includes whether such structures are influenced or generated by the distribution of specific food availability like host plants or other features of their environment (e.g. Stone & Schönrogge 2003, Hayward & Stone 2006).

Current phylogeographic patterns of certain species are not only influenced by historical processes, colonisation events and differences between refugial habitats, but also by differences in dispersal abilities, survival rates, or species-specific ecological requirements (Dawson et al. 2002). Therefore, concordant phylogeographic structure is expected for species closely interacting like symbionts, mutualists and host-parasite systems leading to co-evolution and with it to a co-distribution (Funk et al. 2000, Nieberding et al. 2004, LaJeunesse et al. 2004). However, species living in the same habitat undergoing the same environmental changes should also show similar geographical structuring (Nieberding & Olivieri 2007). But even on a local scale, related species sharing the same habitat and history can differ importantly in their genetic structure (Dawson et al. 2002), which emphasises the influence of ecological traits on geographical structuring. On a broader scale the geographical structure is rather influenced by ecological differences, leading to discordant regional patterns (Perkins 2001, Crandall et al. 2008). Another important aspect leading to discordance in geographical patterns might be the shift to another related host species or partner during times the primary host is not available.

Specialised interacting species depending on the distribution of each other additionally share the same habitat and should therefore be influenced by the same historical processes. On regional scales congruent phylogeographical patterns have been found for vertically transmitted endo-symbionts (Funk et al. 2000, LaJeunesse et al. 2004), parasite-host systems (Nieberding et al. 2004, Hayward & Stone 2006), obligate mutualists (Thompson et al. 2005), and even for symbionts with an intermediate host (Criscione & Blouin 2007).

Similar to parasites are parasitoids bound to their host species. In many cases they are highly specialised to one host species and therefore depend on the host's distribution and survival. As with some parasites, the dependence of the parasitoid on its host is restricted to the larval stage, which is followed by a free-living stage as adult. Contrary to parasites, however parasitoids and their hosts are both insect species which often share similar ecological traits such as size and life cycles. Information about comparative phylogeographies of host-parasitoid systems is rare and provides contrary results. Some

parasitoids show congruent patterns with their host species (Hayward & Stone 2006) others do not (Althoff & Thompson 1999, Johannesen & Seitz 2003). Here, we focus on the phylogeographcial pattern of a host-parasitoid system of one gall forming insect and two of its parasitoid species in Europe.

The genetic structure of many species in Europe is highly influenced by the historical events of the Pleistocene ice ages and associated with the geographical pattern of mountain ranges (Hewitt 1996, Taberlet et al. 1998, Seddon et al. 2002, Schmitt 2009). Many European species survived the ice ages within one or more ice free refugia which are usually located in warmer regions in southern Europe, typically on the Iberian Penninsula, Italy, and the Balkans (Lunt et al. 1998, Santucci et al. 1998, Stauffer et al. 1999, Hewitt 1999). Owing to the isolation of these regions during the glaciation periods, the taxa among these refugia differentiated (Hewitt 1996). With the re-warming of the climate, species expanded their ranges again to northern parts of Europe. Secondary contact of populations from different refugia led to complex genetic suture zones for many European taxa (Taberlet et al. 1998, Hewitt 1999, Schmitt 2007). During the climatic cycles of the Pleistocene, the ranges, occupied by the species, expanded and contracted many times. These multiple re-colonisation events are characterised by extinction and bottleneck events leading to a loss of genetic diversity in the northern populations and resulted in complex intraspecific differentiation of many of the re-colonising taxa (Hewitt 1996, Schmitt 2007).

For host-parasitoid systems we expect parasitoids to follow the range changes of their hosts during climatic fluctuations. If hosts and parasites existed together in subdivided refugia during glaciation periods, we expect similar genetic divergence in host and parasitoid populations owing to the same isolation and subsequent re-colonisation (Stone et al. 2001). Due to the close relationship and similar life cycle, we also expect similar differentiation rates within both species.

To test these assumptions we examined the geographical structures of one cynipid gall wasp *Diplolepis rosae* L. (Hym., Cynipidae) and two dependent parasitoid species in Europe. The cynipid wasp is a univoltine gall maker of conspicuous and multichambered galls which are found on *Rosa* species from several dog rose Sections (Schröder 1967). The galls form the basis of a complex community of one inquiline and at least 12 species of parasitoids and hyperparasitoids (Blair 1944, Redfern, Askew 1992). Two of the most frequent parasitoid species are the endoparasitoid *Orthoplema mediator* Thunb. (Hym. Ichneumonidae) and the ectoparasitoid *Glyphomerus stigma* Fabr. (Hym. Torymidae) (Stille 1984). The three chosen insect species live closely connected in the same habitat, have similar life cycles, and are directly or indirectly dependent on the distribution and history of dog roses. Therefore, similar phylogeographic population structures are expected like found for the oak gall wasp *Andricus kollari* and its chalcid parasitoid *Megastigmus stigmatizans* (Hayward and Stone 2006).

The three chosen insect species show different amounts of infection with different types of *Wolbachia* bacteria (Schilthuizen & Stouthamer 1998). In a wide range of arthropod species intracellular bacteria belonging to the genus *Wolbachia* are common (Werren 1997, Werren et al. 2008) and could manipulate the reproduction of their hosts. These micro-organisms are inherited maternally, transmitted by cytoplasm of egg cells, and alter the reproduction of their hosts in several ways, including cytoplasmic incompatibility, male killing, feminisation, and induction of parthenogenetic development (Stouthamer et al. 1999). Due to modifications in reproduction, *Wolbachia* infection also shows long term impacts on the evolution of host taxa and their genetic structure. Parthenogenesis inducing *Wolbachia* strains could reduce gene flow between populations (Werren et al. 2008) and genetic variability (Plantard et al. 1998). Additionally, the reproductive strategies of the species might be important for the current geographical differentiation because of dispersal and consecutive settlement abilities (Dawson et al. 2002).

In the following we will compare the geographical structure of the gall wasp *D. rosae* with the two dependent parasitoid species *O. mediator* and *G. stigma*. The geographical structure will be assessed by two variable DNA regions, first the *cytochrome oxidase subunit I* (COI) in the mitochondrion (mtDNA) and second the *internal transcribed spacer 2* (ITS 2) in the genome. Additionally we will check for the infection rate with *Wolbachia* bacteria of all three insect species in Europe and discuss geographical patterns.

## 5.2 Material and Methods

*Life cycles*

After emergence female gall wasps of *D. rosae* start ovipositing from May till July (Schröder 1967). They induce the growth of mulitchambered galls on buds, stems and leaves of almost all species of dog roses, genus *Rosa*. During summer the gall-maker *D. rosae* is parasitised by at least five parasitoid species among which *O. mediator* and *G. stigma* are the most common ones (Redfern, Askew 1992). During winter gall wasps and parasitoids hibernate in the galls and pupate in spring.

In Europe *O. mediator* emerges from *D. rosae* galls with a mean frequency of 30% of all parasitoids (Schröder 1967, Randolph 2005). It is endoparasitic and the only ichneumonid species which also attacks other *Diploplepis* species (*Diplolepis spinosissimae*, *Diplolepis mayri*, and *Diplolepis eglanteriae*) (Randolph 2005). With a mean frequency of 20% *G. stigma* is the second most frequent parasitoid species in *D. rosae* galls. It is an ectoparasitoid feeding not only on *D. rosae* larvae but also on the inquiline species *Periclistus brandtii* and other parasitoids (Redfern, Askew 1992). In Europe the primary host

is *D. rosae* but from Canada it is recorded attacking also at least six other *Diplolepis* species (Randolph 2005).

The type and infection rate of *Wolbachia* differs between the three chosen insect species. The gall builder *D. rosae* is infected with *Wolbachia* type I (Schilthuizen & Stouthamer 1998). In the literature male individuals are seldom reported (McCallan 1940, Askew 1960, Schröder 1967, Stille 1984). We found an infection rate of more than 99% in Central Germany (see Chapter 4). Schilthuizen and Stouthamer (1998) documented the infection of *O. mediator* with type II *Wolbachia* though only in one of three sampling sites. *G. stigma* was not infected but occurred in just one sampling site.

*Sampling and DNA Extraction*

We sampled galls of *D. rosae* at different sites in Europe during 2006 and 2007 (Table 5.1). All sampled galls were kept individually outside in plastic pots closed with gauze, until July when most of the inhabitants had emerged. The galls were then dissected. The Inhabitants were determined and stored in 90% alcohol. Because *D. rosae* reproduces parthenogenetically, all the individuals of one gall are expected to be clones. Therefore, only one individual per gall was used for genetic analyses. Total DNA was extracted using spin columns (DNeasy tissue kit, Qiagen, Hilden, Germany) according to the manufacturer's protocol for animal tissue. Prior to extraction the insects were ground in 1.5 ml microfuge tubes using sterile plastic pestles.

*Amplification and Sequencing of DNA fragments*

We amplified and sequenced two DNA fragments, firstly ITS 2 with a length of ca. 700bp and secondly the mitochodrial COI with a length of ca. 650 bp. The ITS 2 fragments of *D. rosae* and *G. stigma* were amplified with the following primers, forward ITS5.8F (5´-GTC CAC GGA TAC AAT TCC CGG ACC-3´; Rokas et al. 2002) and reverse ITS 4 (5´-TCC TCC GCT TAT TGA TAT GC-3´; White et al. 1990). Amplifications were performed by an initial denaturation step at 95°C for 2 min, then 30 cycles with denaturation at 95°C for 30 s, annealing at 55°C for 1 min and extension at 72°C for 1 min. These steps were followed by 10 min extension at 72°C.

Table 5.1. Sampling sites, number of individuals and collector of the gall wasp *Diplolepis rosae* and its most frequent parasitoids *Orthopelma mediator* and *Glyphomerus stigma* in Europe.

| Country | Location | Longitude | Latitude | *D. rosae* | *O. mediator* | *G. stigma* | Leg. |
|---|---|---|---|---|---|---|---|
| Austria | Sandeck | 16.800319 | 47.762479 | 3 | 3 | 1 | R. Brandl |
| | Klagenfurt | 14.333300 | 46.633300 | 1 | 0 | 0 | H. Riegler-Hager; H.J. Wagner |
| | Koralpe | 15.120680 | 46.827430 | 1 | 0 | 0 | M. Brändle |
| Czech Republic | Průhonice | 14.550931 | 50.000958 | 1 | 1 | 0 | Z. Kiesenbauer |
| | Olomouc | 17.251804 | 49.595831 | 2 | 1 | 1 | E. Křístková |
| Denmark | Tversted | 10.182948 | 57.581646 | 3 | 3 | 0 | H. Adsersen |
| | Horsholm | 12.508367 | 55.880395 | 3 | 3 | 0 | H. P. Ravn |
| Finland | Örö | 22.323207 | 59.818693 | 3 | 2 | 0 | A. Albrecht |
| France | Nantes | -1.556744 | 47.216938 | 3 | 3 | 2 | O. Plantard |
| | Chelles | 2.593155 | 48.880504 | 3 | 3 | 0 | A. Kohnen |
| | Sarlat-et-Caneda | 1.215711 | 44.887242 | 3 | 2 | 0 | A. Kohnen |
| | Chateau-Challon | 5.616700 | 46.750000 | 3 | 2 | 0 | A. Kohnen |
| | Perigueux | 0.733300 | 45.200000 | 2 | 0 | 0 | A. Kohnen |
| | Doucier | 5.550000 | 46.183300 | 0 | 2 | 2 | A. Kohnen |
| Germany | Ulm | 9.963067 | 48.420743 | 3 | 1 | 0 | P. Zindl |
| | Darmstadt | 8.673421 | 49.870052 | 2 | 2 | 0 | A. Kohnen |
| | Salzgitter | 10.398056 | 52.112778 | 3 | 3 | 3 | A. Marten |
| | Sellin | 13.692020 | 54.377222 | 1 | 1 | 0 | H. Buhr |
| | Poel | 11.419011 | 53.989685 | 1 | 2 | 0 | H. Buhr |
| Hungary | Matrafured | 19.971108 | 47.827859 | 1 | 1 | 3 | C. Penny |
| Italy | Monte Baldo | 10.800000 | 45.783300 | 2 | 2 | 0 | M. Brändle |
| | Siena | 11.316700 | 43.316700 | 3 | 1 | 3 | P. Castagnini |
| Lithuania | Kaunas | 23.892426 | 54.897010 | 0 | 3 | 0 | P. Ivinskis |

Table 5.1. Continued.

| Country | Location | Longitude | Latitude | *D. rosae* | *O. mediator* | *G. stigma* | Leg. |
|---|---|---|---|---|---|---|---|
| Macedonia | Ohrid | 20.800046 | 41.1186 | 2 | 1 | 2 | S. Trajanovski |
| Netherlands | Haaren | 5.219684 | 51.6008 | 1 | 2 | 0 | J. Wolfs |
| | Meeden | 6.883300 | 53.1167 | 1 | 0 | 0 | J. Bijkerk |
| | Haren | 6.600000 | 53.1667 | 1 | 0 | 0 | J. Bijkerk |
| | Rhenen | 5.566700 | 51.9500 | 1 | 0 | 0 | J. Bijkerk |
| Norway | Lier | 10.277810 | 59.7493 | 3 | 0 | 0 | L. O. Hansen |
| Poland | Baligrod | 22.333300 | 49.4833 | 1 | 0 | 0 | N. Selva |
| Slovakia | Martin | 18.789117 | 49.0189 | 3 | 3 | 3 | S. Götzfried |
| Spain | Leon | -5.571391 | 42.5998 | 3 | 3 | 3 | V. Castro-González |
| | Huesca | -0.409261 | 42.1405 | 3 | 2 | 2 | J. D. Moreno |
| | Burgos | -3.683300 | 42.3500 | 1 | 0 | 2 | A. Kempel |
| Sweden | Stockholm | 18.065210 | 59.3311 | 1 | 1 | 0 | B. Gruhne |
| | Uppsala | 17.644586 | 59.8586 | 3 | 2 | 0 | J. Nylander |
| UK | Low Hameringham | 0.050000 | 53.1666 | 3 | 1 | 0 | A. Dale |
| | Langford | -2.825467 | 51.4517 | 2 | 3 | 0 | J. Boyd |
| Ukraine | Kiew | 30.523312 | 50.4503 | 3 | 0 | 1 | T. Parshikova |

The same fragment of *O. mediator* was sequenced with the primers ITS 2 F (5´-GGG TCG ATG AAG AAC GCA GC-3´) and ITS 2 R (5´-ATA TGC TTA AAT TCA GCG GG-3´; Wagener et al. 2006). Amplifications were performed by an initial denaturation step at 95°C for 4 min, then 35 cycles with denaturation at 92°C for 1 min, annealing at 51°C for 1 min and extension at 72°C for 90s, followed by 10 min extension at 72°C.

A fragment of COI was sequenced for all three insect species with the following primers, forward LCO (5´-GGT CAA CAA ATC ATA AAG ATA TTG G-3´) and reverse HCO (5´-TAA ACT TCA GGG TGA CCA AAA AAT CA-3´; Folmer et al. 1994). Amplifications were performed by an initial denaturation step at 95°C for 5 min, then 35 cycles with denaturation at 95°C for 30s, annealing for 45s at 45° C for *D. rosae* and at 40°C for *O. mediator* and *G. stigma,* an extension step at 72°C for 1 min. These steps were followed by a 10 min extension at 72°C.

All reactions were carried out in 20 *µ*l reaction volume including 2-5 *µ*l of template DNA, Tris-HCl (pH 8.3) at 10 mM, KCl at 50 mM, $MgCl_2$ at 1.5 mM, dNTP at 80 *µ*M, each primer at 10 *µ*M and 1 unit of *Taq* DNA Polymerase (New England Biolabs, Frankfurt a. Main, Germany). PCR products were visualised on an agarose gel (2%) and stained with ethidium bromide (2 mg/l, 20 min). ITS 2 fragments of *O. mediator* were extracted from the agarose gels using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). All other PCR products were purified using a Qiagen MinElute PCR purification kit and sequenced directly by Sequencing Laboratories Göttingen GmbH, Germany.

Wolbachia *detection*

Presence or absence of *Wolbachia* bacteria was tested with specific primer pairs amplifying ca. 600bp of the *wsp* gene, forward *wsp* 81F (5´-TGG TCC AAT AAG TGA TGA AGA AAC-3´), reverse *wsp* 691R (5´- AAA AAT TAA ACG CTA CTC CA-3´; Braig et al. 1998).

Whether the absence of a PCR product was caused either by the absence of *Wolbachia* bacteria or by a failure in the reaction was checked with a control primer pair. Control primers for *D. rosae* were forward Dr06-F (5´- CTC ATC TCT TCT TCT TAT CTC AG-3´) and reverse Dr06-R (5´-CCC AGG AGA GCA GAG G-3´; Plantard et al. 1998) amplifying a fragment of 180 bp. Control primers for *O. mediator* and *G. stigma* were LCO and HCO as mentioned before (Folmer et al. 1994)). With all PCR reactions a positive (known infected individual) and a negative (water) control was run. Amplifications were performed by an initial denaturation step at 94°C for 3 min, then 35 cycles with denaturation at 94°C for 1 min, annealing at 50°C or 42°C, respectively, for 1 min and extension at 72°C for 1 min. These steps were followed by a 5 min extension at 72°C.

*Phylogenetic analysis*

Editing and alignment of sequences were performed manually using the computer program BioEdit version 7.0.9 (Hall 1999). Haplotype frequencies and diversities were calculated using the program DnaSP version 4.10 (Rozas et al. 2003). The hierarchical likelihood ratio tests and the Akaike information criterion implemented in MODELTEST 3.7 (Posada & Crandall 1998) were used to select appropriate models of sequence evolution including outgroup individuals (for *D. rosae* two individuals of *D. fructum*; for the COI sequences of *O. mediator* one individual of *Pimpla aequalis* from GenBank AF146681; and for COI sequences of *G. stigma* one individual of *Nassonia vitripennis* from GenBank EU746551).

The suggested models were implemented to calculate corrected distances and construct a neighbour joining tree (NJ) and a maximum parsimony (MP) tree using MEGA version 3.1 (Kumar et al. 2004) and PAUP* version 4.0b10 (Swofford 2002). A heuristic search algorithm was used to construct trees for parsimony analysis. To quantify reliability of nodes 1,000 bootstrap replicates were used for NJ trees and 100 for MP trees, respectively.

For the COI sequences of *D. rosae* a minimum spanning network was constructed using TCS version 1.21 with default settings (Clement et al. 2000). To calculate an analysis of molecular variance (AMOVA) with COI sequences of *O. mediator* Arlequin version 3.11 (Excoffier et al. 2005) was used.

## 5.3 Results

*Diplolepis rosae*

The mtDNA sequences of COI were much more variable than the genomic ITS 2 sequences (Table 5.2). Of the ITS 2 sequences only one individual from Denmark showed a deletion of two base pairs. In both fragments we found only one frequent haplotype (ITS 2: No. 23; COI: No. 34) and several haplotypes just once. With three exceptions individuals from the same sampling site carried also the same haplotype.

For COI sequences Modeltest selected the model of Hasegawa, Kishino and Yano (1985) with among site rate variation (Table 5.3). NJ and MP analysis revealed trees with similar topologies (Fig. 5.1). One major clade was frequent in central and northern Europe and also occurred in Spain (Fig. 5.2). In the minimum spanning network all these haplotypes were closely connected to the most frequent haplotype 1 (Fig. 5.2, Appendix Table A3). Individuals from western France near Nantes (Hap 13) were related with individuals from the UK and Denmark, which shared the same haplotype (Hap 8). In the centre of Europe the frequent haplotypes 4 and 7 were distributed from western France to the Ukraine.

Table 5.2. Number of individuals (No.) for every species and gene fragment, length of the fragment, number of variable sites and parsimony informative sites, outgroup species included in phylogenetic analyses, number of haplotypes, haplotype diversity (*H*), and selected evolution model.

| Gen | Species | No. | Length (bp) | variable sites | Parsimony informative | outgroup | # Hap | *H* | Model |
|---|---|---|---|---|---|---|---|---|---|
| ITS 2 | *D. rosae* | 28 | 581 | 4 | | | 6 | | |
| | *O. mediator* | 27 | 891 | 23 | | | 8 | 0.82 | JC |
| | *G. stigma* | 14 | 440 | 1 | | | 3 | | |
| COI | *D. rosae* | 79 | 654 | 32 | 14 | *Diplolepis fructum* (own sequence) | 15 | 0.79 | HKY+G |
| | *O. mediator* | 56 | 662 | 66 | 44 | *Pimpla aequalis* (AF146681) | 23 | 0.9 | K81uf+G+I |
| | *G. stigma* | 28 | 609 | 71 | 33 | *Nassonia vitripennis* (EU746551) | 24 | 0.99 | HKY+G |

Table 5.3. Model parameters for COI sequences

| Species | Model | A | C | G | T | Ti/Tv | α | I | Distances |
|---|---|---|---|---|---|---|---|---|---|
| *D. rosae* | HKY+G | 0.298 | 0.099 | 0.159 | 0.443 | 2.9 | 0.19 | -- | 0.002 - 0.024 |
| *O. mediator* | K81uf+G+I | 0.331 | 0.149 | 0.118 | 0.102 | unequal | 0.72 | 0.58 | 0.002 – 0.062 |
| *G. stigma* | HKY+G | 0.286 | 0.123 | 0.144 | 0.447 | 7.6 | 0.07 | -- | 0.003 – 0.040 |

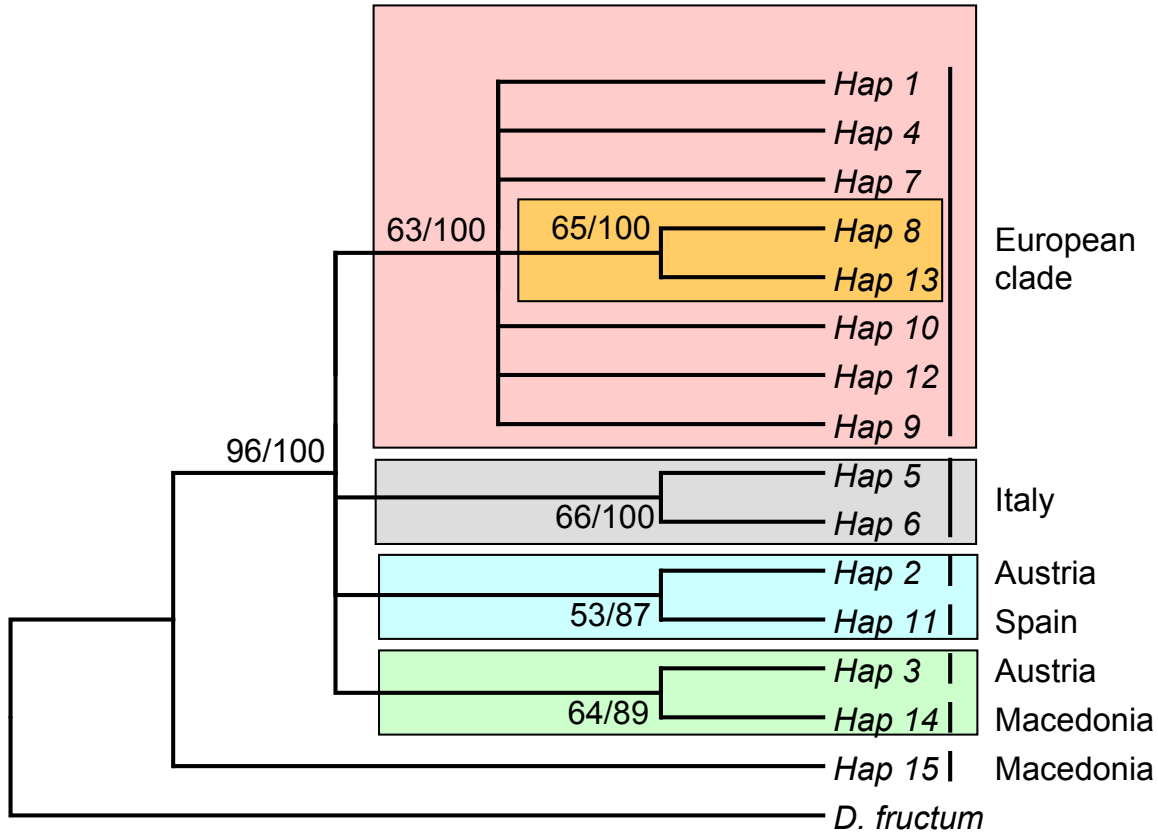


Fig. 5.1. Phylogenetic tree derived by COI haplotypes of *Diplolepis rosae*. First numbers are bootstrap values derived by NJ with 1,000 replicates, second numbers MP with 100 replicates.

Three additional subclades were all located in southern Europe (Fig. 5.1 and Fig. 5.2). One subclade in Spain and Austria was only distantly connected with the most frequent haplotype 1. The two other subclades were more closely connected, one was exclusively found in Italy, the other one in Austria and Macedonia. One second individual from Macedonia (Hap. 15) was very different to all other *D. rosae* individuals and excluded from the minimum spanning network (see Fig. 5.1).

*Orthopelma mediator*

As found for *D. rosae*, COI sequences were much more variable than ITS 2 sequences (Table 5.2) which showed two positions with deletions of one or two base pairs. Two haplotypes (Hap 3 and 5) were most frequent with 8 individuals, respectively (Appendix Table A3). The most frequent haplotype of COI (Hap. 2) was found in 14 individuals, the next frequent were haplotypes 1 and 11 within 8 individuals, respectively. For ITS 2 sequences the program Modeltest selected the Jukes-Cantor model (Jukes & Cantor 1969), and for COI sequences the K81 model (Kimura 1981) with unequal base frequencies (Table 5.3).

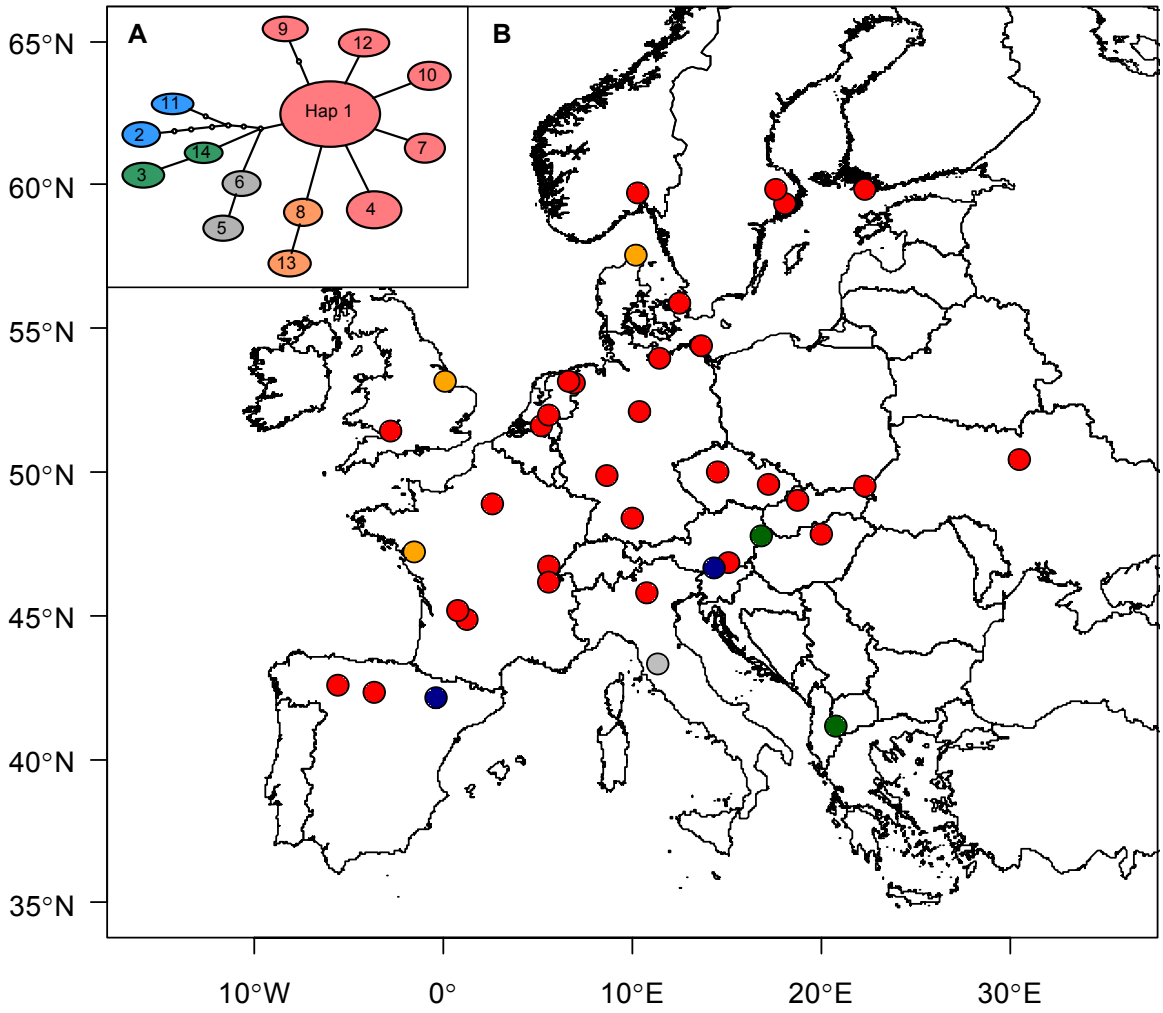


Fig. 5.2. Interconnection and distribution of COI haplotypes of *Diplolepis rosae* in Europe. **A**. Representation of haplotypes by a minimum spanning network, the surface of circles is proportional to the number of individuals bearing this particular haplotype. **B**. Geographical distribution of clades in Europe, without haplotype 15 in Macodonia. Red = major clade, orange = subclade with hap. 8 and 13; blue = subclade with hap. 2 and 11; green = subclade with hap. 3 and 14; grey = subclade with hap. 5 and 6. (See also Fig. 5.1).

All trees showed similar topologies (Fig. 5.3), dividing *O. mediator* species into two major clades in Europe. One clade was found in western Europe distributed from Spain over France, the UK and Denmark up to Sweden. The other clade was found in eastern Europe in Macedonia, central Europe, and Lithuania up to Finland (Fig. 5.4). Three individuals showed incongruent clade affiliation out of which two individuals were from France and one was from northern Germany. With ITS 2 sequences all three were assigned to the Eastern clade, but with COI sequences they belonged to the Western clade, pointing to a hybrid event. With COI sequences one individual from Italy is only distantly related with the two clades. Both

Western and Eastern clades were supported by high bootstrap values and showed low distances within and higher distances between each other (Table 5.4). An AMOVA found only 6% of variation within clades (SS = 53, df = 53), but 94 % between clades (SS = 422, df = 1). The pariwise $F_{ST}$ value between clades was high (0.94; $p < 0.0001$). The haplotype diversity *H* in the Eastern clade was 0.66, but in the Western clade it was much higher (0.91).

Table 5.4. Mean corrected distances between clades of *Orthopelma mediator* derived by COI and ITS 2 sequences (see also Fig. 5.3)

| | COI | | ITS2 | |
|---|---|---|---|---|
| | West | East | West | East |
| West | 0.0058 | | 0.0011 | |
| East | 0.0517 | 0.0045 | 0.0138 | 0.0051 |
| Italy | 0.0564 | 0.0617 | | |

*Glyphomerus stigma*

The ITS 2 sequences were extremely similar, only one variable position was found in four individuals from Italy, Macedonia, Ukraine and Czech Republic. In addition the one individual from Ukraine showed a deletion of two base pairs.

In contrast, the COI sequences were highly variable (Table 5.2). Only four haplotypes were found twice, all other haplotypes were found just once (Appendix Table A3). But translated into amino acids only two (of 202) positions were variable. Modeltest selected the HKY+G model (Hasegawa et al. 1985; Table 5.3). In all topologies (Fig. 5.5) one individual from Ukraine (the same which had the deletion in the ITS 2 fragment) was always differentiated from the other haploytpes, with a mean corrected distance of 0.0661. All other haplotypes were clustered in one major clade with bootstrap support of 61%. Within this major clade only two significant subclades were found, one in central Europe, the other one in Spain. But both subclades did not include all haplotypes of the accordant geographical region.

*Wolbachia* infection

*Wolbachia* infection was very common in the gall wasp *D. rosae*. Of 79 individuals, 10 were not infected, so more than 86% were infected. Additionally both outgroup individuals of *D. fructum* from the Ukraine were also infected. We found no clear geographical pattern of infection (Fig. 5.6 A).

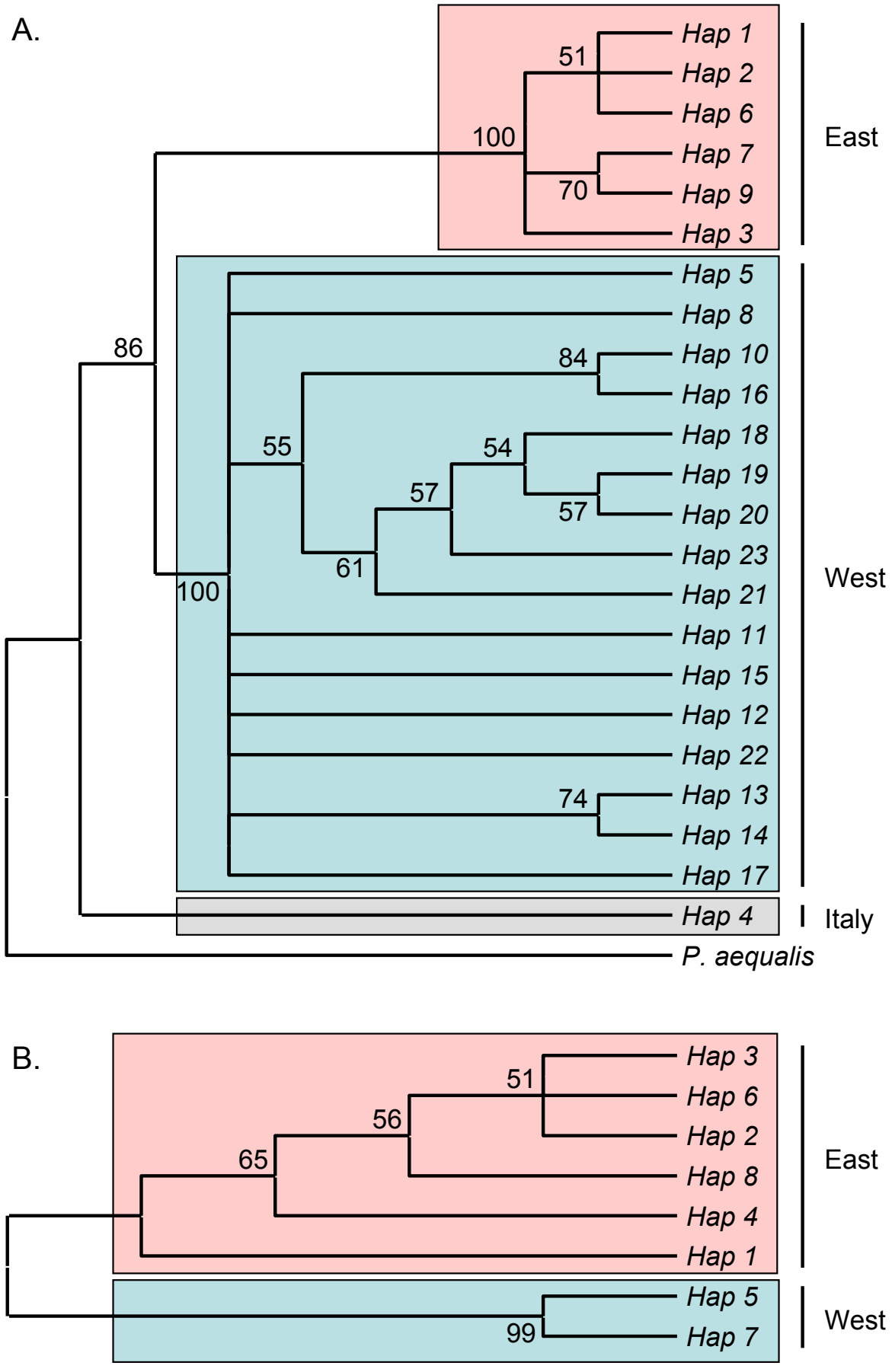


Fig. 5.3. Phylogenetic tree of *Orthopelma mediator,* **A**. NJ tree derived with sequences of COI. **B**. UPGMA tree with ITS 2 sequences.

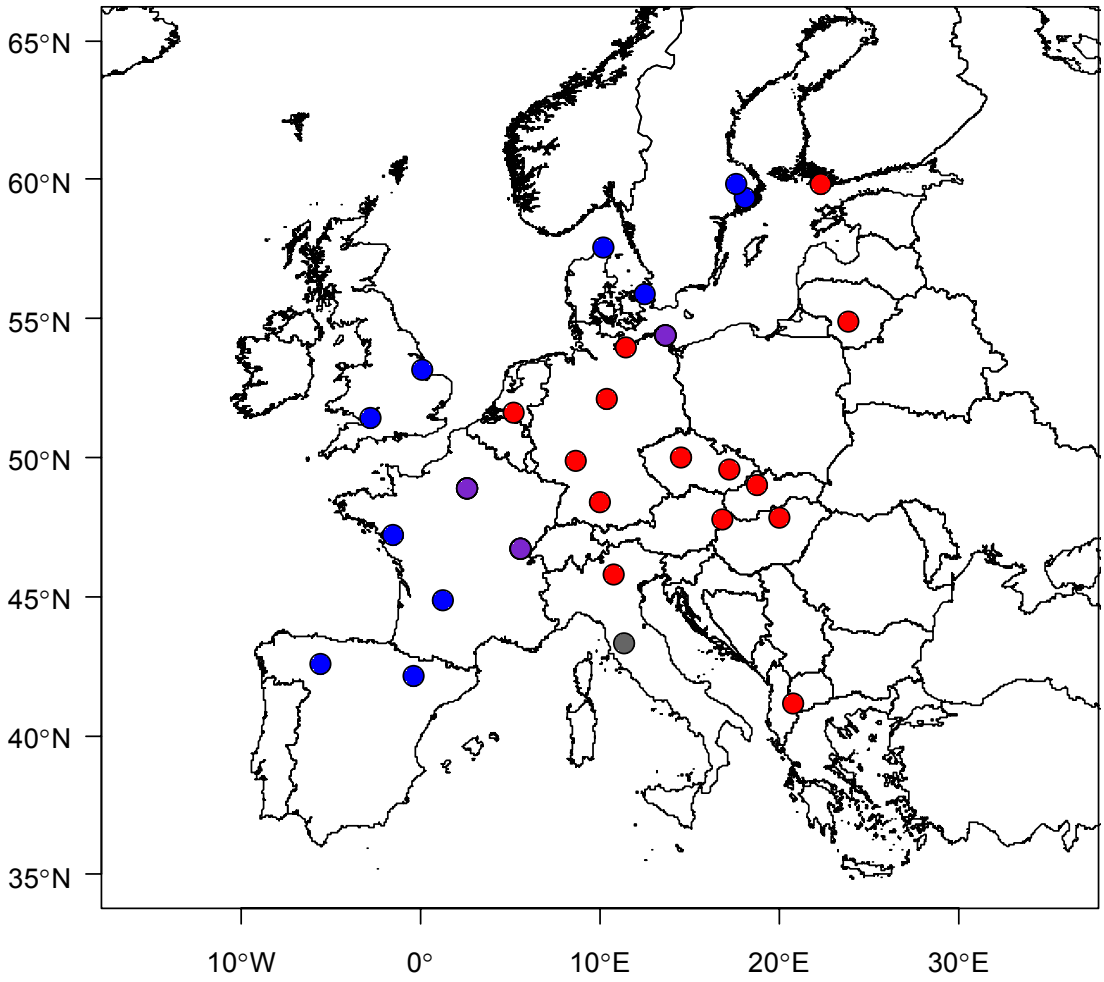


Fig. 5.4. Geographical distribution of the Eastern and Western clades derived by COI and ITS 2 seqeunces of *O. mediator* in Europe. Blue = West clade, red = East clade, grey = haplotype in Italy, only found with COI sequences. At the purple sampling sites individuals were assigned different with the two markers, with ITS 2 to the Eastern clade, but with COI to the Western clade.

The most common parasitoid *O. mediator* was much less infected with *Wolbachia* bacteria. We found 18 infested individuals out of 56, which is an infection rate of 32%. Populations with infected individuals were distributed all over Europe except in Scandinavia (Fig. 5.6 B). In the other parasitoid species *G. stigma* not one single individual (No. 28) was infested with *Wolbachia* bacteria (Fig. 5.6 C).

## 5.4 Discussion

For the gall-parasitoid system of the gall wasp *D. rosae* and two of its most common parasitoids *O. mediator* and *G. stigma*, we expected similar phylogeographic population structures as found in other galling systems (Hayward & Stone 2006).

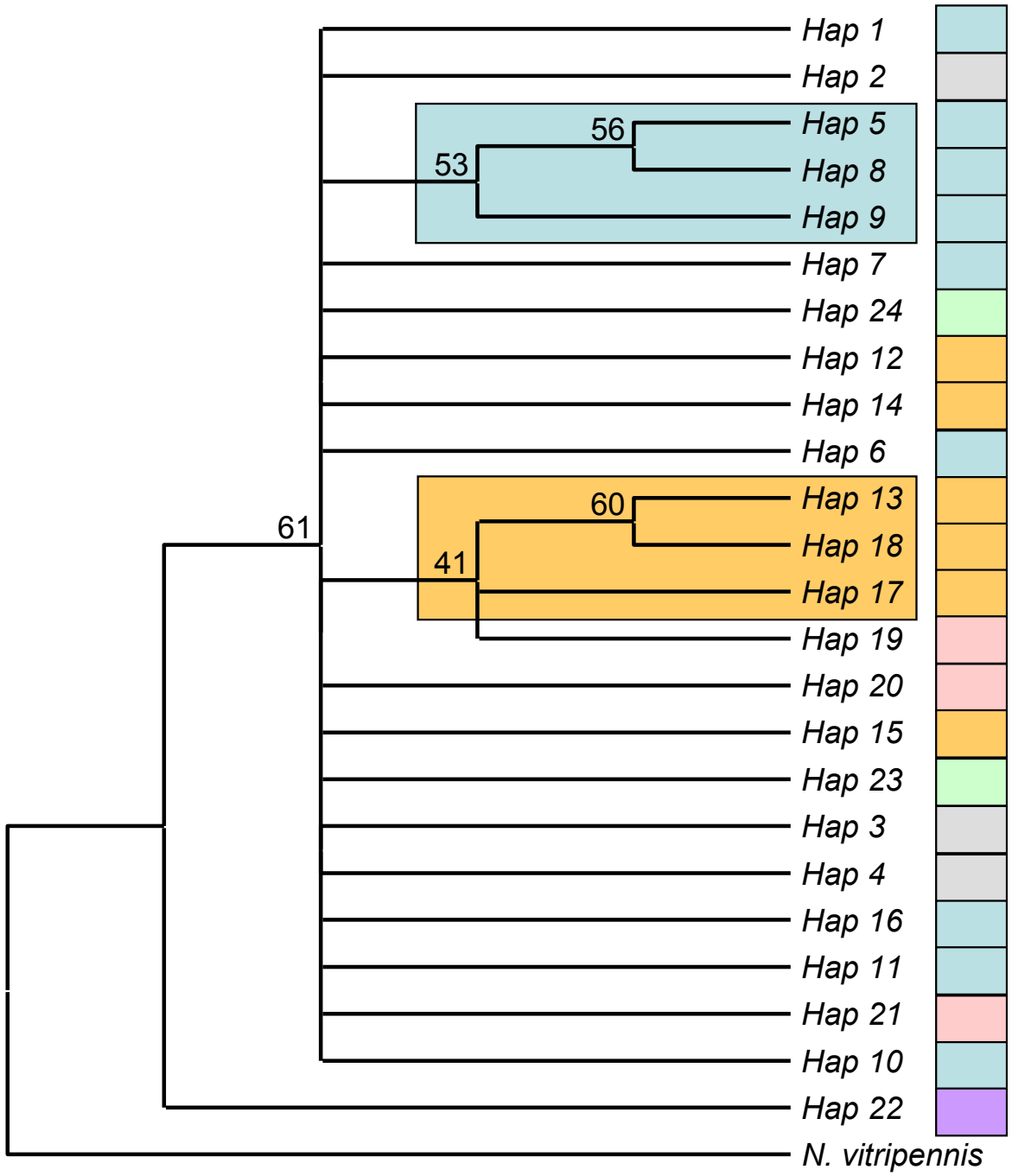


Fig. 5.5. Phylogenetic tree constructed with COI sequences of *G. stigma* haplotypes. Blue = central Europe including samplings from Germany, Hungary, Austria, Czech Republic and Slovakia, grey = Italy, green = Macedonia, yellow = Spain, red = France, purple = Ukraine (see also Fig. 5.6 C).

However, the three insect species were not structured on similar spatial scales. Accordingly, we found no congruence in geographical patterns as well as in levels of gene diversity. Similar results were found by Althoff and Thompson (1999) who found incongruent patterns in population structure between two moth species and their parasitoids in North America. For the fruit fly *Urophora cardui* and its primary parasitoid *Eurytoma robusta* Johannesen and

Seitz (2003) found congruent patterns of genetic differentiation only on a local scale but independent structures globally.

*Geographic structure of the host,* Diplolepis rosae

The gall wasp *D. rosae* showed low haplotype diversity and low genetic distances, compared to the two parasitoid species. The widespread of the major haplotype points to high gene flow between sampling sites. Gene flow in *D. rosae* would mean exchange of individuals between sites, because of the parthenogenetic life cycle. Therefore, no genetic exchange due to reproduction and recombination could occur between individuals. High gene flow within *D. rosae* would support the assumption that *D. rosae* has good dispersal abilities. This gall wasp is a small facile insect easily blown away by winds.

As documented for other European species with higher haplotype diversities in southern regions of Europe, all subclades were restricted to Southern Europe. This is in concordance with the expectation of Pleistocene refugia in Southern Europe, isolated during cold periods and leading to higher diversity within such regions (Hewitt 1996, Taberlet et al. 1998). Two re-colonisation routes would be possible, one from Spain, where the central European clade is also found, or a re-colonisation from eastern Europe down to Spain. But without time estimations we do not want to speculate about possible refugia or re-colonisation routes.

*Geographic structure of the parasitoid species,* Orthopelma mediator *and* Glyphomerus stigma

In contrast to the gall wasp the parasitoid species showed higher haplotype diversities and at least *O. mediator* has a clear geographical structure. We found a pronounced divergence into two lineages - an Eastern and a Western clade - as found in many European plant and animal species (Taberlet et al. 1998, Hewitt 1999). Usually, this is supposed to be the result of Pleistocene survival in isolated refugia located in Southern Europe and following re-colonisation routes into central Europe. Usually re-colonising populations from these restricted refugia managed to cross barriers like the Pyrenees and the Caucasus mountains whereas Italian populations were often unable to cross the Alps (Taberlet et al. 1998). Both *O. mediator* lineages showed such a classical distribution of clades and a high divergence between lineages. In central areas, where both clades live in sympatry, a suture zone is found which is shown by three “hybrid” individuals from France and Germany. They belong to different clades depending on which marker gene is analysed.

In the other parasitoid species, *G. stigma,* we did not find such a clear geographical pattern.

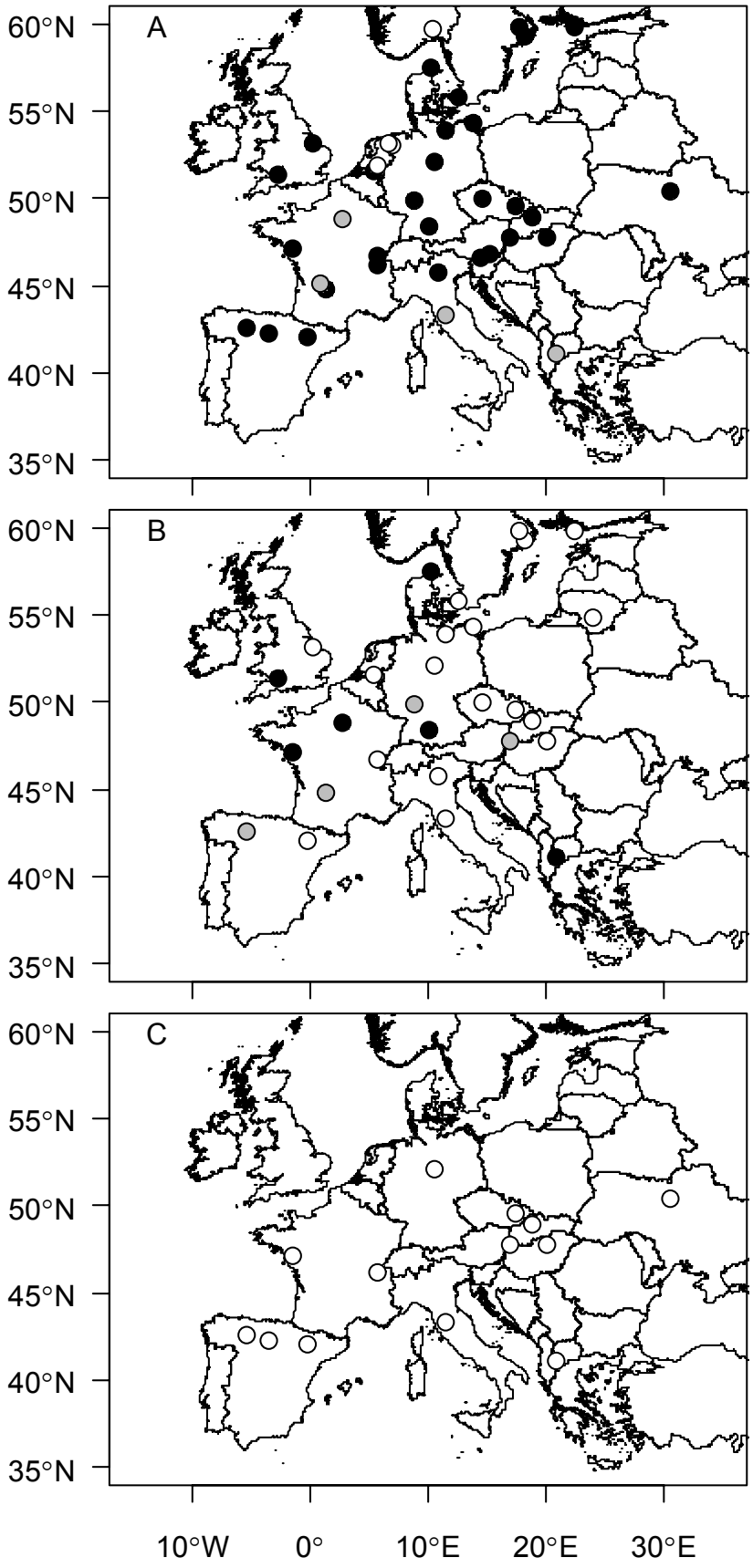


Fig. 5.6. Distribution of *Wolbachia* infection in **A**. *Diplolepis rosae* **B**. *Orthopelma mediator* and **C**. *Glyphomerus stigma*. Black = populations with complete infection, grey = partial infection, white = no infection.

This may point to a high amount of mixing and exchange between populations and therefore also to good dispersal abilities. In the literature it is noted that *G. stigma* seems to depend not only on the distribution of the host species but is also limited by latitude. It was found, that *G. stigma* is absent in the North of England (Randolph 2005) and Scandinavia (Nordlander 1973, Stille 1984). Accordingly, we found no gall in Scandinavia infested with *G. stigma* but a high amount in southern regions. Even in Britain galls exhibited no *G. stigma*. In Canada however *G. stigma* is recorded as a common parasitoid of *D. rosae* and even other *Diplolepis* species (Randolph 2005). Therefore, the restriction in distribution by latitude in Europe is not apparent in North America and possibly has no climatic causes.

### Wolbachia *infection*

*Wolbachia* bacteria are normally vertically transmitted from mother to offspring with the cytoplasm of egg cells. Some phylogenetic studies have shown that host and symbiont phylogenies are incongruent (Stouthamer et al. 1993, Schilthuizen & Stouthamer 1997) which suggests that horizontal transmission must have occurred frequently. For the *D. rosae* system Schilthuizen and Stouthamer (1998) found no horizontal transmission between gall wasps and parasitoids, because host and parasitoids are infected with different types or strains of *Wolbachia*. Accordingly, we found no congruent geographical infection structure between host and parasitoids. The infection rates varied between an almost complete infection of *D. rosae,* a partial infection of *O. mediator* and no infection of *G. stigma* individuals. In *O. mediator* some populations of both geographical lineages were infected. This could be explained by several transmissions which have occured, at least one in every lineage from an unknown source. As mentioned above a transmission from *D. rosae* is not possible because it carries another type of *Wolbachia* bacteria. However, an infection by one of the other *Diplolepis* species which are also parasitised by *O. mediator* would be possible.

The infection with *Wolbachia* had no a clear impact on any of the insect species' phylogeny, or explained a geographical pattern. Genetic diversity however was inversely linked with *Wolbachia* infection. The species with the lowest diversity, *D. rosae*, showed the highest infection.

### *Comparing geographical structure of host and parasitoids*

Contrary to parasite-host systems which often show congruent patterns in phylogeography, even at regional scales (Funk et al. 2000, Nieberding et al. 2004, Thompson et al. 2005, Criscione & Blouin 2007), parasitoids are bound to their host species only during larval stage. One parasitoid larva consumes its host completely which leads to the death of the host larva.

After imaginal ecdysis the parasitoids are forced to search for new hosts for their offspring. During this time the strict bond between host and parasitoid is repealed and both species have the opportunity to disperse independently. Different dispersal abilities and subsequent colonisation success could influence population structures, altering population histories over time scales, and disentangle population structures of similar species (Dawson et al. 2002). Nevertheless, parasitoids' dispersal can be only successful as long as they are within the distributional range of available hosts.

Therefore, a main factor in separating population structures should be the ability of parasites or parasitoids to switch between host species. It would be a great advantage for a species to have the possibility to switch onto another host species should the primary host be locally rare or even extinct. Both the gall wasp and the parasitoids are not restricted to one single host species, but they are dependent on a certain host genus. The most common host plants of *D. rosae* are dog roses of the section Caninae. Dog roses cover more than 60 species which hybridise and differ in several characters. Not much is known about their history beyond that this section originated by hybridisation and colonised Europe after the last glacial retreat (Wissemann 2002, Ritz et al. 2005b). Afterwards a re-colonisation of Europe from eastern regions is expected (Dingler 1907). Today the distribution and density of roses is highly influenced by humans. Many species and cultivars are planted in parks, gardens and along roadsides all over central Europe. New varieties are produced by natural hybridisations, which later spread and potentially are introduced into other countries. Nevertheless, most of the natural dog roses show different distributional ranges and somewhat different habitat requirements. By infesting almost all lineages of dog roses the gall wasp is able to colonise the whole palaearctic region.

As gall inducing insects, parasitoids are usually highly specialised to one species or at least one genus. Both parasitoid species in our study are dependent on gall inducing species of the genus *Diplolepis*, which causes galls on rose species (Randolph 2005).

*O. mediator* is reported from three other *Diplolepis* species, *D. spinosissimae*, *D. eglanteria*, and *D. mayri*. The majority of the other *Diplolepis* species build galls much smaller than *D. rosae* galls, mostly with just one chamber for one larva. Furthermore, some *Diplolepis* species such as *D. spinosissimae* are restricted to one host plant species, *Rosa spinossisima* L. The other parasitoid *G. stigma*, infests not only *D. rosae* but also the inquilin and other parasitoids within *D. rosae* galls. Its infection of other *Diplolepis* species is only recorded from Canada and is unknown in Europe. Therefore, we suppose *D. rosae* to be the main host species for both parasitoids, but the switch between different host species allows the parasitoid species to colonise areas where *D. rosae* is rare. Accordingly, we cannot exclude the probability that the parasitoid species survived during colder periods separated

from their primary host species, and, therefore, the three insect species share not exactly the same history.

*Conclusion*

The differing patterns in geographical distribution of the three closely connected insect species can be explained by two processes. Firstly, the parasitoid species are not bound to their hosts during their whole life time, as adults they have the possibility to disperse separately which leads to discordant spatial patterns. Secondly, the possibility to switch to another host species, if the primary host is not available, may cause discordance between geographical structures of the gall wasp and its parasitoids.

## ACKNOWLEDGEMENTS

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# 6. No genetic differentiation in the rose-infesting fruit flies *Rhagoletis alternata* and *Carpomya schineri* (Diptera: Tephritidae) across central Europe

Annette KOHNEN, Volker WISSEMANN, and Roland BRANDL

ABSTRACT

After the last glacial retreat in Europe, multiple recolonizations led to intraspecific differentiation in many of the recolonizing taxa. Here we investigate the genetic diversification across central Europe in two recolonizing taxa, the tephritid fruit flies *Rhagoletis alternata* (Fallén 1814) and *Carpomya schineri* (Loew 1856), which attack rose hips. Analysis of amplified and sequenced fragments of the mitochondrial genes encoding cytochrome oxidase I (800 bp), cytochrome oxidase II (470 bp) and cytochrome *b* (450 bp), indicate that all the individuals of *Rh. alternata* (n = 21) collected from across Europe share the same haplotype. Two individuals of *C. schineri* from Berlin, which is further north of the range than previously reported in the literature, differ from the other individuals (n = 13) in one nucleotide position on the cytochrome oxidase II gene fragment. This level of genetic variation in sequences with a summed length of 1720 bp is unexpectedly lower than in other insect taxa (n= 63). This might have been caused by a selective sweep by a cytoplasmic symbiont such as *Wolbachia,* or a recent range expansion associated with a host shift or a single recolonization event.

## 6.1 Introduction

Many European species survived the last ice age in one or several refugia. Owing to the isolation of these regions during this period, the taxa in the refugia differentiated (Hewitt 1996). With the warming of the climate, species ranges expanded again and populations from different refugia came into secondary contact (Hewitt 1996), which resulted in many taxa in Europe having complex genetic suture zones (Taberlet et al. 1998).

Here, we analyse the genetic diversification in two tephritid fruit flies that attack rose hips: *Rhagoletis alternata* (Fallén) and *Carpomya schineri* (Loew). The former, *Rh. alternata* is distributed throughout the Palaearctic region (Kandybina 1977, Smith & Bush 2000). In contrast, *C. schineri* has a more restricted distribution in southern Europe, southern Asia and northern Africa (White & Elson-Harris 1992). In southern Europe, these two species occur sympatrically. The larvae of both fruit flies develop in rose hips, where they feed not on the seeds but on the hypanthium. Adult females of *Rh. alternata* lay their eggs under the surface of hips, which they mark with a pheromone that deters other females. Around October, the larvae leave the hips and pupate in the soil (Bauer 1986, 1998). The larvae of *C. schineri* leave the hips around August and the adult flies emerge around June the following year (Hendel 1927).

Both flies are host specific at the generic level and their distribution depends on that of their host plants, mainly the dog roses, *Rosa* section *Caninae*. Members of this rose section are the most common rose species in Europe and western Asia. They are evolutionarily young, originated by hybridization (Wissemann 2002, Ritz et al. 2005b) and expanded their range into central and northern Europe after the last ice age (Dingler 1907). The distribution of the roses provided a platform for the flies to recolonize Europe from certain refugia. If the flies came from Mediterranean refugia there should be genetic suture zones in central Europe. We therefore screened the genetic variation in samples of flies collected from populations across this area.

## 6.2 Material and methods

*Collection of larvae*

Infested rose hips were collected in September 2004 from the canton Valais in Switzerland and in 2006 across Europe (Fig. 6.1; Appendix: Table A1), sent to our laboratory in perforated plastic bags and then stored at 15 °C. The larvae were allowed to leave the hips and pupate within the bags. Pupae were stored in 95%-alcohol at 5 °C for DNA analysis.

Pupae parasitized by *Utetes magnus* (Fischer 1958) are brown (T. Hoffmeister personal communication), which allowed us to select non-parasitized individuals for DNA extraction. As a control, some adult *Rh. alternata* were allowed to emerge from each sample.

*DNA extraction and amplification of mtDNA fragments*

DNA was extracted from pupae using spin columns (DNeasy tissue kit, Qiagen, Hilden, Germany). Three mitochondrial DNA (mtDNA) fragments were chosen for amplification: 800 bp of cytochrome oxidase I (COI) amplified with primers C1-J-2183 (forward, 5´-CAA CAT TTA TTT TGA TTT TTT GG-3´) and TL-N-3014 (reverse, 5-´TCC ATT GCA CTA ATC TGC CAT ATT A-3´; Simon et al. 1994), 470 bp of cytochrome oxidase II (COII) amplified with primers C2-J-3291 (forward, 5´-GAA ATA ATT TGA ACA ATT CTA CCA GC-3´) and TK-N-3772 (reverse, 5´-GAG ACC ATT ACT TGC TTT CAG TCA TCT-3´; Smith & Bush 1997), and 450 bp of the 3´end of cytochrome *b* (Cyt *b*) amplified with primers CB-J-10933 (forward,

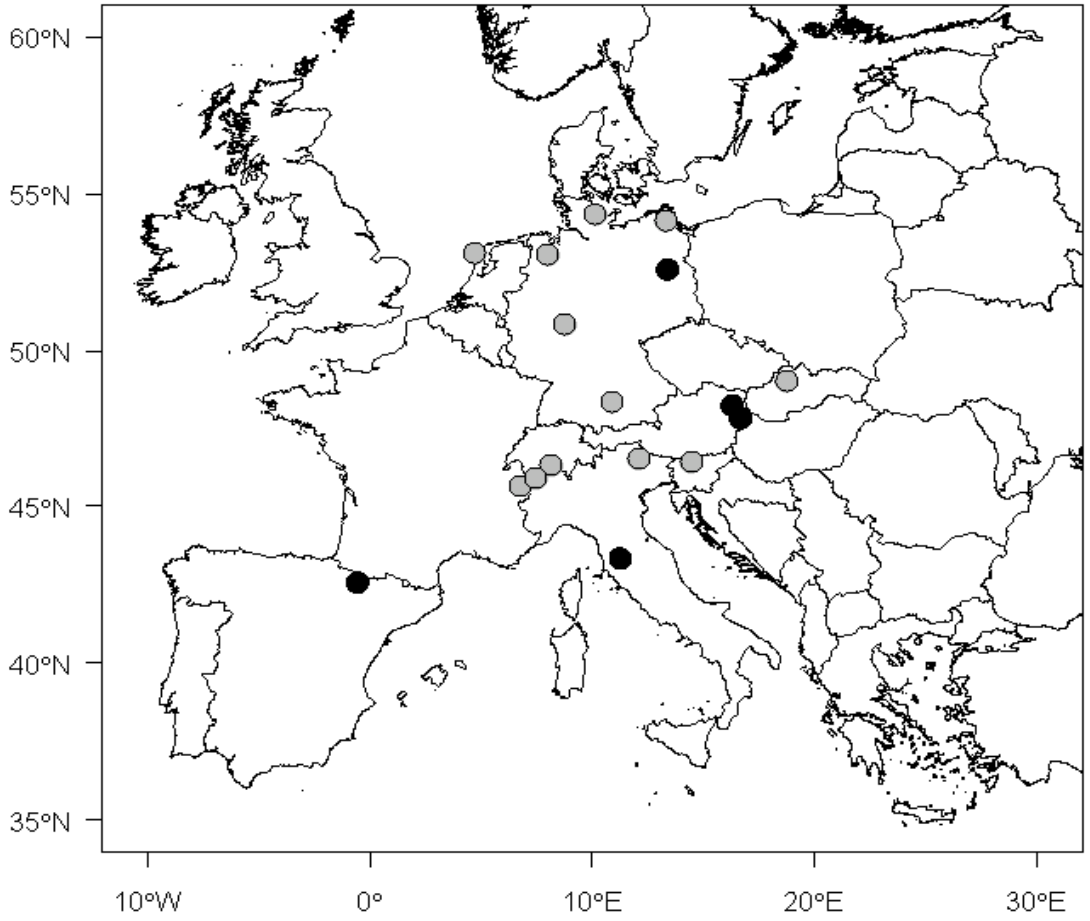


Fig. 6.1. Sites at which *Rhagoletis alternata* (grey circles) and *Carpomya schineri* (black circles) were sampled in Europe.

5´-TAT GTA CTA CCA TGA GGA CAA ATA TC-3´) and CB-N-11367 (reverse, 5´-ATT ACA CCT CCT AAT TTA TTA GGA AT-3´; Simon et al., 1994); all positions as in the mitochondrial genome of *Drosophila yakuba* (Clary & Wolstenholme 1985). A thermocycler (Eppendorf Mastercycler, Hamburg, Germany) was used for the amplifications following the protocol: initial denaturation cycle at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing for 1 min at 60 °C for the COI primers, 58 °C for COII primers, and 45 °C for the Cyt *b* primers and then extension at 72 °C for 1 min. The final extension step was for 10 min at 72 °C.

The amplification reactions (20 µl) contained 20–100 ng of template DNA, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 80 µM dNTP, 10 µM of each of the two primers and 1 unit of *Taq* DNA polymerase (Sigma, Taufkirchen, Germany). The products were purified using a Qiagen MinElute PCR purification kit (Qiagen, Hilden, Germany). The purified PCR products were sequenced directly in both directions by Sequencing Laboratories Göttingen GmbH, Germany.

*Sequence analysis*

We compared the variability in mtDNA with that recorded for other insect populations. We searched for population genetic studies of insect taxa in the ISI Web of Knowledge. The aim of our ISI web search was not to construct a complete data set, but to retrieve sufficient information for statistical analysis. We used the keywords “Insect”, “Diptera”, “Lepidoptera”, “Coleoptera” or “Hymenoptera”, in combination with “genetic structure” and/or “genetic differentiation”. We selected these keywords after some testing. These preliminary searches showed that the selected keyword combinations retrieved sufficient but a manageable number of papers. Furthermore, to obtain the most recent publications only the first 100 were selected. Finally, we excluded studies on parthenogenetic and social insects as they are known to show low genetic variability. From the published reports on these insect taxa, we extracted the number of haplotypes found and the number and length of the sequences (Appendix: Table A2). All variables were $\log_{10}$-transformed before analysis using a general linear model in STATISTICA (Version 6.1). Both fly species were included in the analyses. The number of expected haplotypes was calculated for both fly species using the coefficients of the model (Table 6.1).

Table 6.1. Estimated coefficients and analyses of variance of the general linear model.

| | Estimates | df | SS | MSS | F | *P* |
|---|---|---|---|---|---|---|
| Intercept | –0.765 | 1 | 0.206 | 0.206 | 1.526 | 0.22 |
| log (length of sequence) | 0.232 | 1 | 0.182 | 0.182 | 1.347 | 0.25 |
| log (number of sequence) | 0.744 | 1 | 5.72 | 5.72 | 42.25 | < 0.001 |
| Residuals | | 60 | 8.12 | 0.135 | | |

## 6.3 Results and Discussion

We sequenced the chosen mtDNA fragments of 21 individuals of *Rh. alternata* from 12 localities across central and southern Europe (Fig. 6.1). None of the 1720 nucleotide positions from COI, COII and Cyt *b* (accession numbers FJ571363, FJ571366 and FJ571369, respectively) were variable, i.e. there is only one haplotype throughout the area sampled. The nucleotide sequences of the COII gene fragment were identical to the COII sequence of *Rh. alternata* available in GenBank (acc. no. U53260). We also found no differences in the sequences of the amplified COI (acc. nos. FJ571364 and FJ571365) and Cyt *b* gene fragments (acc. nos. FJ571370 and FJ571371) from 13 individuals of *C. schineri* from 4 localities, and the nucleotide sequences of the COI gene fragment were identical to the published sequence for this species (acc. no. U53267). In contrast to the situation in *Rh. alternata*, the COII gene fragment sequences from two individuals of *C. schineri* (acc. no. FJ571367) differed at one nucleotide position from those in the other 11 individuals (acc. no. FJ571368), which shared one haplotype. The two individuals with the second haplotype were collected in Berlin, far north of the range of *C. schineri* reported in the literature (Kandybina 1977, White & Elson-Harris 1992, Smith & Bush 2000).

The mtDNA genes chosen for our analyses, those encoding cytochrome oxidase I and II subunits and cytochrome *b,* are protein-encoding genes with considerable variability even between closely related species and populations of the same species (Rokas et al. 2000, Simon et al. 1994). Furthermore, the chosen fragments of these genes include the most variable positions of the genes and are therefore often used for population genetic studies of animals, especially insects (e.g. Lunt et al. 1998, Rokas et al. 2003, Arias et al. 2005, Pramual et al. 2005, Sezonlin et al. 2006). These criteria indicate that the selected mtDNA gene fragments should be suitable for elucidating the biogeographic history of these fly species.

We compared our findings with mtDNA sequence data for other insect populations (Fig. 6.2). For 21 sequences with a summed length of 1720 bp (Fig. 6.2) 9–10 expected haplotypes were calculated and 6–7 haplotypes for 13 sequences of the same summed length. The standardized residual of the model for *Rh. alternata* was -2.93 ($P$ = 0.0016) and for *C. schineri* –1.62 ($P$ = 0.053).

Compared to the results for other insect populations, the genetic variation in *Rh. alternata* and *C. schineri* was significantly lower than expected. This could have at least two explanations. One possibility is that symbionts, such as *Wolbachia,* shape mtDNA evolution (Hurst & Jiggins 2005), which would constrain the suitability of mtDNA sequences for molecular biogeographic studies of insects. During the initial phase of symbiont invasion, selective sweeps may reduce mtDNA diversity, thereby producing a genetic signal similar to that produced by a population bottleneck with subsequent expansion (Hurst & Jiggins 2005). *Wolbachia* is known to infect members of the genus *Rhagoletis* (Riegler & Stauffer 2002) but not members of the genus *Carpomya* (Kittayapong et al. 2000). The second possibility is that the flies recently and rapidly expanded their range from one source population. Such an expansion could be induced by colonization or a host-shift; in both cases, lower levels of genetic variation would be expected due to founder effects (Harrison 1991). Both of the fly species studied are specialists and therefore dependent on the distribution of their host, members of the genus *Rosa* section *Caninae.* These dog roses originated by hybridization

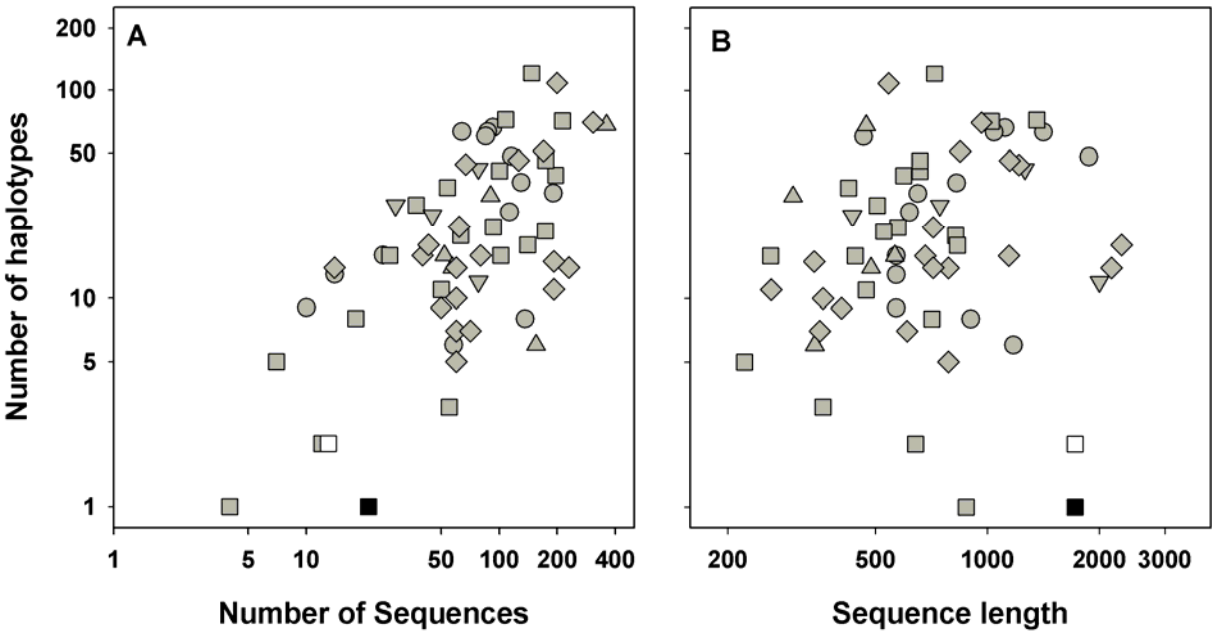


Fig. 6.2. Number of haplotypes in relation to **A**. the number of sequences analysed (all variables $\log_{10}$ transformed) and **B**. sequence length for 63 insect taxa (Supplementary material obtained from the literature cited in Appendix Table A2). Twenty-one sequences from *Rhagoletis alternata* (black square) and 13 from *Carpomya schineri* (white square) are included in this analysis. Grey squares, Diptera; grey circles, Coleoptera; grey triangles, Hymenoptera; grey diamonds, Lepidoptera; grey inverted triangles, other insect orders.

during the last ice age (Wissemann 2002, Ritz et al. 2005b) prior to the recolonization of Europe (Dingler 1907). Founder individuals of the two fly species may have shifted to this new host, which would provide an explanation for the low genetic variability.

Today, the distribution and density of roses is influenced by humans. *Rosa rugosa,* for example, was introduced from East Asia about 100 years ago (Hegi 1975) and is now cultivated in parks, gardens and along roads all over Central Europe. Also its rose hips are attacked by *Rh. alternata*, and this increase in available hosts may have triggered the spread of these flies across Europe. *Rh. alternata* disperses well, and even the Alps do not seem to be an effective geographical barrier (Vaupel et al. 2007). This increase in distribution can also be partly explained by the behaviour of the females. After oviposition, the females mark the rose hips with a pheromone (Bauer 1986, 1998). Often, a high proportion of the hips, up to 100%, are infested and marked. Females leave such locations and search for rose shrubs with a lower proportion of infested hips. These observations and our results indicate that a recent range expansion of the flies from an unknown source area may account for their low genetic variability. Nuclear markers of *Rh. alternata*, e.g. allozyme genes, also show little variability, which suggests a high level of gene flow between European populations (Leclaire & Brandl 1994, Vaupel et al. 2007). Note also that our finding *C. schineri* near Berlin extends the known range of this species northwards. The range expansion of this fly species could also be influenced by humans, who may have transported the larvae in plant material.

In conclusion, we found a surprisingly low level of genetic variability in tephritid fruit fly populations across central Europe. The reasons remain uncertain, but a recent and single colonization from an unknown source or the host shift to *Rosa* section *Caninae* are plausible explanations. The reporting of such findings is likely to facilitate a pluralistic understanding of the biogeography of plants and animals living in Europe.

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# 7. Summary

The insect fauna associated with a particular plant species depends on the available species pool of phytophages, the distribution and abundance of the host species, the number of feeding niches as well as the host's taxonomic isolation and biochemical make-up. Variations in plant traits can influence the abundance of herbivorous insects as well as the structure and dynamics of associated herbivore communities (e.g. Cattell & Stiling 2004, Bailey et al. 2006). Even plant traits encoded by few genes may have important effects on the community of plant exploiters, as suggested by the concept of the "extended phenotype" (Whitham et al. 2003). Radiation processes in plant species provide a variety of new plant traits in a short time period and morphological differences in closely related species. Differences in morphological traits could result in host preferences of herbivorous insects and, as a consequence, in adaptations to certain host species. These adaptations may lead to genetically differentiated populations of exploiters living in sympatry (Drès & Mallet 2002). Therefore, the most important question in interacting systems is: How do radiation and diversity of the hosts translate into the radiation and diversity of the exploiters?

Similarly important for the understanding of interactions between species is the understanding of geographical structuring of host and exploiter species. This includes to which extent interacting species like parasitoid and host populations are structured on similar spatial scales and whether such structures are influenced or generated by the distribution of specific food availability like host plants or other features of their environment (Stone & Schönrogge 2003, Hayward & Stone 2006).

Dog roses and their exploiters were chosen as model system to study the effects of a rapid radiation of hosts on dependent herbivores because they underwent a recent radiation process during the Pleistocene and are widespread in Europe. Three common members of the dog rose section *Caninae* (DC.) Ser. (*Rosa canina* L., *R. corymbifera* Borkh. and *R. rubiginosa* L.) were selected for our investigations. The available information on phytophages on roses shows that there is a large number of generalists attacking roses, but also many specialists for example the cynipid wasp *Diplolepis rosae* L. (Hym.) and the tephritid flies *Rhagoletis alternata* (Fallén 1814) and *Carpomya schineri* (Loew 1856). For host-specific interacting species, assumably connected by a co-evolution and co-distribution,

not only host preferences and following host adaptations but also concordant phylogeographic structures are expected (Funk et al. 2000, Nieberding et al. 2004, LaJeunesse et al. 2004). Due to the close relationship and similar life cycle, we also expect similar differentiation rates within both species.

Within the dog rose system we analysed (1) host preferences of *D. rosae* and its associated community, (2) host-associated genetic differentiation of *D. rosae*, (3) comparative phylogeographies of *D. rosae* and two of its most common parasitoids, and (4) genetic differentiation of *Rh. alternata* and *C. schineri*.

*(1) Cynipid gall-parasitoid interactions, comparing three dog rose species along a geographical gradient (Chapter 3)*

To investigate host preferences of the gall wasp *D. rosae* or its associated parasitoid community the abundances of wasp and parasitoids on three dog rose species were determined along a geographical gradient in Germany. Within 388 galls we found eight species of parasitoids (most common: *Orthopelma mediator* 18%, *Glyphomerus stigma* 12%, *Torymus bedeguaris* 9.1%) and the inquiline *Periclistus brandtii* (12%). High variations in gall densities as well as in parasitism rates at different geographical sites were found. In the same way the gall volume and the communities associated with *D. rosae* galls varied at different geographical sites. With increasing gall volume the rate of parasitism decreased due to the length of the parasitoid's ovipositor. In contrast to our expectations, the host species had only a minor influence on gall densities or parasitoid communities. Most important for host choice was the habit of the shrub especially the availability of developing shoots. Nevertheless, due to significant interactions, the host species in combination with the geographical site had a complex impact on the abundance of exploiter densities and their dependent community.

*(2) No host-associated differentiation in the gall wasp* Diplolepis rosae *(Hymenoptera: Cynipidae) on three dog rose species (Chapter 4)*

Because the plant tissue of dog roses is manipulated by *D. rosae* during gall formation, a close evolutionary relationship between the wasp and different rose species is expected which may lead to a genetic adaptation of *D. rosae* to certain dog rose species. Additionally, the parthenogenetic reproduction of *D. rosae* induced by strains of the bacterial genus *Wolbachia* could influence both the host-associated genetic structure and geographic patterns. We found that almost 100% of the *D. rosae* individuals are infected with *Wolbachia* sp. Using 106 polymorphic AFLP markers we found no genetic differentiation among the wasps from different host plants and also from different geographic locations.

*(3) Comparing geographical structures of one cynipid gall wasp with two specialised parasitoids in Europe (Chapter 5)*

Similar phylogeographic population structures for host-parasitoid-pairs were expected because they share the same environmental and ecological influences. We compared the geographical structures in Europe of *D. rosae* and two of its most common parasitoid species *O. mediator* and *G. stigma.* Two gene fragments were sequenced: The mitochondrial cytochomre oxidase I (650 bp) and the genomic internal transcribed spacer 2 (700bp). An additional factor influencing current geographical structures might be the infection with *Wolbachia* bacteria which alter the reproduction strategy and therefore the dispersal ability of its host. In contrast to our expectations, the geographical structure of the three species was incongruent. The gall wasp had the lowest genetic diversity with one major central clade, *O. mediator* showed a classical European distribution with one eastern and one western clade, whereas *G. stigma* had the highest diversity but no geographical structuring. The infection rate with *Wolbachia* bacteria however showed the opposite behaviour, it was highest in *D. rosae* with 86%, intermediate in *O. mediator* with 32% and lowest in *G. stigma* which was not infected at all.

*(4) No genetic differentiation in the rose-infesting fruit flies* Rhagoletis alternata *and* Carpomya schineri *(Diptera: Tephritidae) across central Europe (Chapter 6)*

The genetic diversification across central Europe in two re-colonising taxa, the tephritid fruit flies *Rh. alternata* and *C. schineri*, which attack rose hips, revealed no geographical structuring. Analysis of amplified and sequenced fragments of the mitochondrial genes encoding cytochrome oxidase I (800 bp), cytochrome oxidase II (470 bp) and cytochrome *b* (450 bp), indicated that all the individuals of *Rh. alternata* (n = 21) collected from all across Europe shared the same haplotype. Two individuals of *C. schineri* from Berlin, which is further north of the range than previously reported in the literature, differed from the other individuals (n = 13) in one nucleotide position on the cytochrome oxidase II gene fragment. This level of genetic variation in sequences with a summed length of 1720 bp is unexpectedly lower than in other insect taxa (n= 63).

Overall, we conclude that the ongoing hybridisation within the genus *Rosa* may act as a “hybrid bridge”, preventing preferences and adaptations of exploiters to one specific host plant. The lack of geographical structuring of *D. rosae* could be explained by large population sizes and the good dispersal ability combined with low genetic variation owing to *Wolbachia* infection. The main reasons for the discordance of geographical patterns between parasitoids and host insect might be the free living stage as adults with different possibilities

to disperse and the ability to switch to another host, if the primary host is not available. The same might be true for the fruit flies. The low genetic differentiation might be caused by a selective sweep by a cytoplasmic symbiont such as *Wolbachia,* or a recent range expansion associated with a host shift or a single re-colonisation event. Thus, the genetic variability and diversity of dog roses is not translated into a host-specific radiation process of the dependent insect fauna.

# 8. Referenzen

**Adler H (1877)** Beiträge zur Naturgeschichte der Cynipiden. *Deutsche Entomologische Zeitschrift* **21:** 209-248.

**Althoff DM, Thompson JN (1999)** Comparative geographic structures of two parasitoid-host interactions. *Evolution* **53:** 818-825.

**Althoff DM, Thompson JN (2001)** Geographic structure in the searching behaviour of a specialist parasitoid: combining molecular and behavioural approaches. *Journal of Evolutionary Biology* **14**: 406-417.

**Alvarez-Castellanos PP, Bishop CD, Pasqual-Villalobos MJ (2001)** Antifungal activity of the essential oil of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. *Phytochemistry* **57:** 99-102.

**Andres MA, Connor EF (2003)** The community-wide and guild specific effects of pubescence on the folivorous insects of manzanitas *Arctostaphylos* spp. *Ecological Entomology* **28:** 383-396.

**Arias L, Bejarano EE, Marquez E, Moncada J, Velez I, Uribe S (2005)** Mitochondrial DNA divergence between wild and laboratory populations of *Anopheles albimanus* Wiedemann (Diptera: Culicidae). *Neotropical Entomology* **34**: 499-506.

**Askew RR (1960)** Some observations on *Diplolepis rosae* (L.) (Hym., Cynipidae) and its parasites. *Entomologist`s monthly magazine* **95:** 191-192.

**Bagatto G, Paquette C, Shorthouse JD (1996)** Influence of galls of *Phanacis taraxaci* on carbon partitioning within common dandelion, *Taraxacum officinale*. *Entomologia Experimentalis et Applicata* **79:** 111-117.

**Bahçecioğlu Z, Yildiz B (2005)** A study on the microfungi of Sivas Province. *Turkish Journal of Botany* **29:** 23-44

**Bailey JK, Wooley SC, Lindroth RL, Whitham TG (2006)** Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecology Letters* **9:** 78-85.

**Barratt EM, Gurnell J, Malarky G, Deaville R, Bruford MW (1999)** Genetic structure of fragmented populations of red squirrel (*Sciurus vulgaris*) in the UK. *Molecular Ecology* **8:** 55-63.

**Barrowclough GF, Groth JG, Mertz LA, Gutierrez RJ (2005)** Genetic structure, introgression, and a narrow hybrid zone between northern and California spotted owls (*Strix occidentalis*). *Molecular Ecology* **14:** 1109-1120.

**Bauer G (1986)** Life history strategy of *Rhagoletis alternata* (Diptera: Tephritidae), a fruit fly operating in a “non-interactive” system. *Journal of Animal Ecology* **55:** 785-794.

**Bauer G (1998)** Structure and function of a non-interactive insect-plant system. *Oecologia* **115:** 154-160.

**Bayer MH (1992)** Biochemical modification of the phenotype in cynipid galls. In: Williams MAJ (ed) Plant Galls Organisms, Interactions, Population, Clarendon Press, Oxford, pp 429-446.

**Blair KG (1944)** A note on the economy of the rose bedeguar gall, *Rhodites rosae*, L. *Proceedings and Transactions of the South London Entomological and Natural History Society* **44:** 55-59.

**Bonin A, Bellemain E, Eidesen PB, Pompanon F, Brochmann C, Taberlet P (2004)** How to track and assess genotyping errors in population genetics studies. *Molecular Ecology* **13:** 3261-3273.

**Braig HR, Zhou WG, Dobson SL, O'Neill SL (1998)** Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. *Journal of Bacteriology* **180:** 2373-2378.

**Brandenburger W (1994)** Die Verbreitung der in den westlichen Ländern der Bundesrepublik Deutschland beobachteten Rostpilze (Uredinales). *Regensburger Mykologische Schrirften* **3:** 1-381.

**Brandl R, Vidal S (1987)** Ovipositor length in parasitoids and tentiform leaf mines - Adaptations in eulophids (Hymenoptera, Chalcidoidea). *Biological Journal of the Linnean Society* **32:** 351-355

**Brandl R, Mann W, Sprinzl M (1992)** Estimation of the monocot-dicot age through tRNA sequences from the chloroplast. *Proceedings of the Royal Society of London, Series B* **249:** 13 – 17.

**Brändle M, Brandl R (2001)** Species richness of insects and mites on trees: expanding Southwood. *Journal of Animal Ecology* **70:** 491-504.

**Bronner R (1992)** The role of nutritive cells in the nutrition of cynipids and cecidomyiids. In: Shorthouse JD, Rohfritsch O (eds) Biology of insect-induced galls. Oxford University Press, New York, pp 118-140.

**Bruneau A, Starr JR, Joly S (2007)** Phylogenetic relationships in the genus *Rosa*: new evidence from chloroplast DNA sequences and an appraisal of current knowledge. *Systematic Botany* **32:** 366-378.

**Brunner PC, Chatzivassiliou EK, Katis NI, Frey JE (2004)** Host-associated genetic differentiation in *Thrips tabaci* (Insecta; Thysanoptera), as determined from mtDNA sequence data. *Heredity* **93:** 364-370.

**Bush GL (1969)** Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). *Evolution* **23:** 237-251.

**Bush GL (1992)** Host race formation and sympatric speciation in *Rhagoletis* fruit flies (Diptera: Tephritidae). *Psyche* **99:** 335-357.

**Cakir A, Kordali S, Zengin H, Izumi S, Hirata T (2004)** Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*. *Flavour and Fragrance Journal* **19:** 62-68.

**Carisio L. Cervella P. Palestrini C. DelPero M. Rolando A. (2004)** Biogeographical patterns of genetic differentiation in dung beetles of the genus *Trypocopris* (Coleoptera, Geotrupidae) inferred from mtDNA and AFLP analyses. *Journal of Biogeography* **31**: 1149-1162.

**Cattell MV, Stiling P (2004)** Tritrophic interactions and trade-offs in herbivore fecundity on hybridising host plants. *Ecological Entomology* **29:** 255-263.

**Clarke GM, Whyte LS (2003)** Phylogeography and population history of the endangered golden sun moth (*Synemon plana*) revealed by allozymes and mitochondrial DNA analysis. *Conservation Genetics* **4**: 719-734.

**Clary DO, Wolstenholme DR (1985)** The mitochondrial DNA molecule of *Drosophila yakuba*: Nucleotide sequence, gene organization, and genetic code. *Journal of Molecular Evolution* **22**: 252-271.

**Clay K (1989)** Clavicipataceous fungal endophytes of grasses coevolution and the change from parasitism to mutualism. In: Pirozynski KA, Hawksworth, DL (eds.): Coevolution of fungi with plants and animals. Academic Press, London, pp 79-106.

**Clement M, Posada D, Crandall KA (2000)** TCS: a computer program to estimate gene genealogies. *Mol.Ecol.* **9:** 1657-1659.

**Coates BS, Sumerford DV, Hellmich RL (2004)** Geographic and voltinism differentiation among North American *Ostrinia nubilalis* (European corn borer) mitochondrial cytochrome c oxidase haplotypes. *Journal of Insect Science* **4**: Art no 35.

**Crandall ED, Jones ME, Munoz MM, Akinronbi B, Erdmann MV, Barber PH (2008)** Comparative phylogeography of two seastars and their ectosymbionts within the Coral Triangle. *Molecular Ecology* **17:** 5276-5290.

**Crawley MJ, Long CR (1995)** Alternate bearing, predator satiation and seedling recruitment in *Quercus robur*. *Journal of Ecology* **83:** 683-696.

**Criscione CD, Blouin MS (2007)** Parasite phylogeographical congruence with salmon host evolutionarily significant units: implications for salmon conservation. *Molecular Ecology* **16:** 993-1005.

**Da Costa-da-Silva AL, Capurro ML, Bracco JE (2005)** Genetic lineages in the yellow fever mosquito *Aedes* (*Stegomyia*) *aegypti* (Diptera: Culicidae) from Peru. *Memorias do Institutio Oswaldo Cruz* **100**: 639-644.

**Dallas JF, Cruickshank RH, Linton Y-M, Nolan DV, Patakakis M, Braverman Y, Capela R, Capela M, Pena I, Meiswinkel R, Ortega MD, Baylis M, Mellor PS, Mordue AJ (2003)** Phylogenetic status and matrilineal structure of the biting midge, *Culicoides imicola*, in Portugal, Rhodes and Israel. *Medical and Veterianry Entomology* **17**: 379-387.

**Dawson MN, Louie KD, Barlow M, Jacobs DK, Swift CC (2002)** Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. *Molecular Ecology* **11:** 1065-1075.

**Diegisser T, Johannesen J, Lehr C, Seitz A (2004)** Genetic and morphological differentiation in *Tephritis bardanae* (Diptera: Tephritidae): evidence for host-race formation. *Journal of Evolutionary Biology* **17:** 83-93.

**Dingler H (1907)** Versuch einer Erklärung gewisser Erscheinungen in der Ausbildung und Verbreitung der wilden Rosen. *Mitteilungen des Naturwissenschaftlichen Vereins zu Aschaffenburg* **6:** 1-38.

**Doebley J (2004)** The genetics of maize evolution. *Annual Reviews of Genetics* **38**: 37-59.

**Drès M, Mallet J (2002)** Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **357:** 471-492.

**Eber S, Knoll S, Brandl R (1999)** Endophagous insects and structural niches on plants: ecology and evolutionary consequences. *Ecological Entomology* **24:** 292-299.

**Ehrlich PR, Rave H (1964)** Butterflies and plants: a study in co-evolution. *Evolution* **18:** 586-608.

**Eigner A, Wissemann V (1999)** *Rosa* X *mangii*, eine neue intersektionelle Hybride charakterisiert durch morphologische und genetische Untersuchungen. *Haussknechtia* **7:** 35-40.

**Eisenbach J (1996)** Three-trophic-level interactions in cattail hybrid zones. *Oecologia* **105**: 258-265.

**Eisner T, Eisner M, Hoebeke ER (1998)** When defense backfires: Detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. *Proceedings of the National Academy of Science* **95:** 4410-4414.

**El-Gazzar A (1981)** Chromosome numbers and rust susceptibility as taxonomic criteria in *Rosaceae*. *Plant Systematics and Evolution* **137:** 23-38.

**Emerson BC, Orami P, Hewitt GM (2000)** Tracking colonization and diversification of insect lineages on islands: mitochondrial DNA phylogeography of *Tarphius canariensis* (Coleoptera: Colydiidae) on the Canary Islands. *Proceedings of the Royal Society of London Series B* **267**: 2199-2205.

**Espirito-Santo MM, Neves FDS, Andrade-Neto FR, Fernandes GW (2007)** Plant architecture and meristem dynamics as the mechanisms determining the diversity of gall-inducing insects. *Oecologia* **153:** 353-364.

**Esseghir S, Ready PD, KillickKendrick R, Benlsmail R (1997)** Mitochondrial haplotypes and phylogeography of *Phlebotomus* vectors of *Leishmania major. Insect Molecular Biology* **6**: 211-225.

**Evanno G, Regnaut S, Goudet J (2005)** Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14:** 2611-2620.

**Evans KJ, Jones MK, Mahr FA, Roush RT (2000)** DNA phenotypes of the blackberry biological control agent, *Phragmidium violaceum*, in Australia. *Australasian Plant Pathology* **29:** 249-254.

**Excoffier L, Smouse PE, Quattro JM (1992)** Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131:** 479-491.

**Excoffier L, Laval G, Schneider S (2005)** Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* 47-50.

**Feder JL, Chilcote CA, Bush GL (1988)** Genetic differentiation between sympatric host races of the apple maggot fly *Rhagoletis pomonella*. *Nature* **336:** 61-64.

**Ferrari J, Kruess A, Tscharntke T (1997)** Auswirkungen der Fragmentierung von Rosenbüschen auf deren Insektenlebensgemeinschaften (Effects of rosebush fragmentation on insect communities). *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie (Bayreuth 1997)* **11:** 87-90.

**Feuerhahn B, Spethmann W (1995)** Kreuzungen bei Wildrosenarten. Gehölzforschung 3, Institut für Obstbau und Baumschule, Hannover.

**Floate KD, Whitham TG (1993)** The „hybrid bridge“ hypothesis: host shifting via plant hybrid swarms. *American Naturalist* **141:** 651-662.

**Foley DH, Torres EP (2006)** Population structure of an island malaria vector. *Medical and Veterianry Entomology* **20**: 393-401.

**Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994)** DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3:** 294-299.

**Frenzel M, Brandl R (1998)** Diversity and composition of phytophagous insect guilds on Brassicaceae. *Oecologia* **113:** 391-399.

**Fricke R (2004)** Vergleich der Lebensgemeinschaften von Wirbellosen auf drei nahe verwandten Wildrosenarten (Sektion *Caninae*) über einen geographischen Gradienten. Diplomarbeit, Allgemeine Ökologie und Tierökologie, Philipps-Universität Marburg, Germany.

**Fritz RS, Price PW (1988)** Genetic variation among plants and insect community structure: willow and sawflies. *Ecology* **69:** 845-856.

**Fritz RS, Nichols-Orians CM, Brunsfeld SJ (1994)** Interspecific hybridization of plants and restistance to herbivores: hypotheses, genetics, and variable responses in a diverse herbivore community. *Oecologia* **97:** 106-117.

**Fritz RS, McDonough SE, Rhoads AG (1997)** Effects of plant hybridization on herbivore-parasitoid interactions. *Oecologia* **110:** 360-367.

**Fryer G, Iles TD (1972)** The cichlid fishes of the great lakes of Africa: Their biology and evolution. Edinburgh, Oliver & Boyd.

**Funk DJ, Helbling L, Wernegreen JJ, Moran NA (2000)** Intraspecific phylogenetic congruence among multiple symbiont genomes. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267:** 2517-2521.

**Gange AC (1995)** Aphid performance in an alder (*Alnus*) hybrid zone. *Ecology* **76:** 2074-2083.

**Gäumann E (1959)** Rostpilze Mitteleuropas mit besonderer Berücksichtigung der Schweiz. Büchler, Bern.

**Grand PR (1986)** Ecology and evolution of Darwin's finches. Princeton, NJ: Princeton University Press.

**Gustafsson A (1944)** The constitution of the *Rosa canina* Complex. *Hereditas* **30:** 405-428.

**Gustafsson AP, Hakansson A (1942)** Meiose in some Rosa-hybirds. *Bot. Notiser* **95**: 331-334.

**Hall TA (1999)** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp.Ser.* **41:** 95-98.

**Halverson K, Heard SB, Nason JD, Stireman JO (2008)** Differential attack on diploid, tetraploid, and hexaploid *Solidago altissima* L. by five insect gallmakers. *Oecologia* **154:** 755-761.

**Harper LJ, Schönrogge K, Lim KY, Francis P, Lichtenstein CP (2004)** Cynipid galls: insect-induced modifications of plant development create novel plant organs. *Plant, Cell and Environment* **27:** 327-335.

**Harrison RG (1991)** Molecular changes at speciation. *Annual Review of Ecology and Systematics* **22:** 281-308.

**Hartley SE (1998)** The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall-former? *Oecologia* **113:** 492-501.

**Harvey JA, Van Dam NM, Gols R (2003)** Interactions over four trophic levels: foodplant quality affects development of a hyperparasitoid as mediated through a herbivore and its primary parasitoid. *Journal of Animal Ecology* **72:** 520-531.

**Hasegawa M, Kishino H, Yano T (1985)** Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22:** 160-174.

**Hayward A, Stone GN (2006)** Comparative phylogeography across two trophic levels: the oak gall wasp *Andricus kollari* and its chalcid parasitoid *Megastigmus stigmatizans*. *Molecular Ecology* **15:** 479-489.

**He L, Watabe H, Xiangyu J, Gao J Liang X, Aotsuka T, Zhang Y (2007)** Genetic differentiation and cryptic speciation in natural populations of *Drosophila lacertosa*. *Molecular Phylogenetics and Evolution* **43**: 24-31.

**Hegi G (1975)** *Illustrierte Flora Mitteleuropa* IV/2C. Parey, München, p. 50.

**Hendel F (1927)** Trypetidae. In Lindner E (eds). *Die Fliegen der paläarktischen Region Bd. V*. Schweizerbart, Stuttgart, pp 91-93.

**Hewitt GM (1996)** Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58:** 247-276.

**Hewitt GM (1999)** Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* **68:** 87-112.

**Hewitt GM (2000)** The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907-913.

**Hilker M, Rohfritsch O, Meiners T (2002)** The plant's response towards insect egg deposition. In: Hilkers M, Meiners T (eds) Chemoecology of insect eggs and egg deposition. Blackwell Scientific Publications, Berlin, pp. 205-233.

**Hochwender CG, Fritz RS (2004)** Plant genetic differences influence herbivore community structure: evidence from a hybrid willow system. *Oecologia* **138:** 547-557.

**Hodkinson ID (1997)** Progressive restriction of host plant exploitation along a climatic gradient: the willow psyllid *Cacopsylla groenlandica* in Greenland. *Ecological Entomology* **22:** 47-54.

**Hufbauer RA, Bogdanowicz SM, Harrison RG (2004)** The population genetics of a biological control introduction: mitochondrial DNA and microsatellite variation in native and introduced populations of *Aphidus ervi*, a parasitoid wasp. *Molecular Ecology* **13**: 337-348.

**Hundsdoerfer AK, Wink M (2006)** Incongruence of morphology and genetic markers in *Hyles tithymali* (Lepidoptera: Sphingidae) from the Canary Islands. *Journal of Zoological Systematics and Evolutionary Research* **44**: 316-322.

**Hurst GDD, Jiggins FM (2005)** Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society of London, Series B, Biological Science* **272:** 1525-1534.

**Hutchison DW, Tempelton AR (1999)** Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53:** 1898-1914.

**Johannesen J, Seitz A (2003)** Comparative population genetic structures of the fruit fly *Urophora cardui* and its primary parasitoid *Eurytoma robusta*. *Entomologia Experimentalis et Applicata* **108:** 149-157.

**Johnson MTJ, Agrawal AA (2005)** Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology* **86:** 874-885.

**Johnstone RA, Hurst GDD (1996)** Maternally inherited male-killing microorganisms may confound interpretation of mitochondrial DNA variability. *Biological Journal of the Linnean Society* **58:** 453-470.

**Joly S, Bruenau A (2006)** Incorporating allelic variation for reconstructing the evolutionary history of organisms from multiple genes: an example from *Rosa* in North America. Systematic Biology **55:** 623-636.

**Joly S, Starr JR, Lewis WH, Bruenau A (2006)** Polyploid and hybrid evolution in roses east of the Rocky Mountains. *Amererican Journal of Botany* **9:** 412-425.

**Jukes T, Cantor C (1969)** Evolution of protein molecules. Munro Mammalian Protein Metabolism. New York: Academic Press. 21-132.

**Kandybina MN (1977)** Larvae of fruit-infesting fruit flies (Diptera: Tephritidae). *Opredeliteli po faune SSSR* 153-156.

**Kato K, Hijii N (1997)** Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hym., Cynipidae) on the growth of chestnut trees. *Journal of Applied Entomology* **121:** 9-15.

**Kimura M (1981)** Estimation of evolutionary distances between homologous nucleotide sequences. *Proc.Natl.Acad.Sci.U.S.A* **78:** 454-458.

**Kittayapong P, Milne JR, Tigvattananont S, Baimai V (2000)** Distribution of the reproduction-modifying bacteria, *Wolbachia*, in natural populations of tephritid fruit flies in Thailand. *Science Asia* **26**: 93-103.

**Klášterská I (1969)** Cytology and some chromosome numbers of Czechoslovak Roses I. *Folia Geobotanica et Phytotaxonomica* **4:** 175-189.

**Klášterská I (1971)** Une contribution au probleme de la reproduction sexuelle chez le roses de la section *Caninae*. *Ann Univ et A.R.E.R.S*. **9:** 140-44.

**Klášterská I, Natarajan AT (1974)** Cytological studies of the genus *Rosa* with special reference to the section *Caninae*. *Hereditas* **76:** 97-108.

**Klinge K (2005)** Pflanzen-Herbivore-Parasitoid Interaktionen auf Wildrosenarten und ihren Hybriden entlang eines geographischen Gradienten. PhD-Theses, Agroecology, University of Göttingen, Germany.

**Kohnen A, Wissemann V, Brandl R (2009)** No genetic differentiation of rose-infesting fruit flies *Rhagoletis alternata* and *Carpomya schineri* (Diptera: Tephritidae) across central Europe. *European Journal of Entomology* **106:** 315-321.

**Koptur S (1985)** Alternative defence against herbivores in Inga (Fabaceae: Mimosidae) over an elevational gradient. *Ecology* **66:** 1629-1650.

**Kovarik A, Werlemark G, Leitch AR, Souckova-Skalicka K, Lim YK, Khaitova L, Koulkalova B, Nybom H (2008)**. The asymmetric meiosis in pentaploid dog roses (*Rosa* sect. *Caninae*) is associated with a skewed distribution of rRNA gene families in the gametes. *Heredity* **101:** 359-367.

**Kumar S, Tamura K, and Nei M (2004)** MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* **5**: 150-163.

**Laffin RD, Langor DW, Sperling FAH (2004)** Population structure and gene flow in the white pine weevil, *Pissodes strobi* (Coleoptera: Curculionidae). *Annals of the Entomological Society of Amerika* **97**: 949-956.

**LaJeunesse TC, Bhagooli R, Hidaka M, DeVantier L, Done T, Schmidt GW, Fitt WK, Hoegh-Guldberg O (2004)** Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. *Marine Ecology-Progress Series* **284:** 147-161.

**Lawton JH (1986)** Surface availability and insect community structure: effects of architecture and fractal dimension of plants. In: Juniper B, Southwood R (eds) Insects and the plant surface, Edward Arnold, London, pp. 317-331.

**Leather SR (1986)** Insect species richness of the British Rosaceae: the importance of host range, plant architecture, age of establishment, taxonomic isolation and species-area relationships. *Journal of Animal Ecology* **55:** 841-860.

**Leclaire M, Brandl R (1994)** Phenotypic plasticity and nutrition in a phytophagous insect - Consequences of colonizing a new host. *Oecologia* **100:** 379-385.

**Leebens-Mack J, Pellmyr O (2004)** Patterns of genetic structure among populations of an oligophagous pollinating yucca moth (*Tegeticula yuccasella*). *Journal of Heredity* **95**: 127-135.

**Lewter J A, Szalanski AL, Nagoshi RN, Meagher RL, Owens CB, Luttrell RG (2006)** Genetic variation within and between strains of the fall armyworm *Spodoptera frugiperda*. The *Florida Entomologist* **89**: 63-68.

**Li J, Zhao F, Choi YS, Kim I, Sohn HD, Jin BR (2006)** Genetic variation in the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) in China inferred from mitochondrial COI gene sequence. *European Journal of Entomology* **103**: 605-611.

**Liljeblad J, Ronquist F (1998)** A phylogenetic analysis of higher-level gall wasp relationships (Hymenoptera: Cynipidae). *Systematic Entomology* **23:** 229-252.

**Lim KY, Werlemark G, Matyasek R, Bringloe JB, Sieber V, El Mokadem H, Meynet J, Hemming J, Leitch AR, Roberts AV. (2005)** Evolutionary implications of permanent odd polyploidy in the stable sexual, pentaploid of *Rosa canina* L. *Heredity* **94:** 501-506.

**Long Y, Wan H, Yan F, Xu C, Lei G, Li S, Wang R (2006)** Glacial effects on sequences divergence of mitochondrial COII of *Polyura eudamippus* (Lepidoptera: Nymphalidae) in China. *Biochemical Genetics* **44**: 361-377.

**Lozier JD, Roderick GK, Mills NJ (2007)** Genetic evidence from mitochondrial, nuclear, and endosymbiont markers for the evolution of host plant associated species in the aphid genus *Hyalopterus* (Hemiptera: Aphididae). *Evolution* **61**: 1353-1367.

**Lunt DH, Zhang DX, Szymura JM, Hewitt GM (1996)** The insect cytochrome oxidase I gene: Evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology* **5:** 153-165.

**Lunt DH, Ibrahim KM, Hewitt GM (1998)** MtDNA phylogeography and postglacial patterns of subdivision in the meadow grasshopper *Chorthippus parallelus*. *Heredity* **80:** 633-641.

**Maddox GD, Root RB (1987)** Resistance to 16 diverse species of herbivorous insects within a population of goldenrod; *Solidago altissima*: genetic variation and heritability. *Oecologia* **72:** 8-14.

**Malmstrom J, Christophersen C, Frisvad JC (2000)** Secondary metabolites characteristic of *Penicillium citrinum*, *Penicillium steckii* and related species. *Phytochemistry* **54:** 301-309.

**Mardulyn P, Milinkovitch MC (2005)** Inferring contemporary levels of gene flow and demographic history in a local population of the leaf beetle *Gonioctena olivacea* from mitochondrial DNA sequence variation. *Molecular Ecology* **14**: 1641-1653.

**Maroja LS, Bogdanowicz SM, Wallin KF, Raffa KF, Harrison RG (2007)** Phylogeography of spruce beetles (*Dendroctonus rufipennis* Kirby) (Curculionidae: Scolytinae) in North America. *Molecular Ecology* **16**: 2560-2573.

**Marquis RJ, Whelan C (1996)** Plant morphology, and recruitment of the third trophic level: Subtle and little-recognized defenses? *Oikos* **75:** 330-334.

**McCallan E (1940)** On the occurrence of males of *Rhodites rosae* (L.) (Hymenoptera, Cynipidae). *Proceedings of the Royal Entomological Society of London (A)* **15:** 21-26.

**McPheron BA, Smith DC, Berlocher SH (1988)** Microgeographic genetic variation in the apple maggot *Rhagoletis pomonella*. *Genetics* **119:** 445-451.

**Mirol PM, Schäfer MA, Orsini L, Routtu J, Schlötterer C, Hoikkala A, Butlin RK (2007)** Phylogeographic patterns in *Drosophila montana*. *Molecular Ecology* **16**: 1085-1097.

**Moon DC, Stiling P (2000)** Relative importance of abiotically induced direct and indirect effects on a salt-marsh herbivore. *Ecology* **81:** 470-481.

**Müller C, Agerbirk N, Olsen CE, Boevé J-L, Schafner U, Brakefield PM (2001)** Sequestration of host plant glucosinolates in the defensive hemolymph of the sawfly *Athalia rosae*. *Journal of Chemical Ecology* **27:** 2505-2514.

**Mun J, Bohonak AJ, Roderick GK (2003)** Population structure of the pumpkin fruit fly *Bactrocera depressa* (Tephritidae) in Korea and Japan: Pliocene allopatry or recent invasion? *Molecular Ecology* **12**: 2941-2951.

**Nardi F, Carapelli A, Dallai R, Roderick GK (2005)** Population structure and colonization history of the olive fly, *Bactrocera oleae* (Diptera, Tephritidae). *Molecular Ecology* **14**: 2729-2738.

**Nieberding C, Morand S, Libois R, Michaux JR (2004)** A parasite reveals cryptic phylogeographic history of its host. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271:** 2559-2568.

**Nieberding CM, Olivieri I (2007)** Parasites: proxies for host genealogy and ecology? *Trends in Ecology & Evolution* **22:** 156-165.

**Nordlander G (1973)** Parasitsteklar i galler av *Diplolepis rosae* (L.) och *D. mayri* Schlechtd. (Hym. Cynipidae) (Hym. Ichneumonoidea, Chalcidoidea, Cynipidea). *Ent.Tidskr* **94:** 148-176.

**Nybom H, Esselink GD, Werlemark G, Vosman B (2004)** Microsatellite DNA marker inheritance indicates preferential pairing between two highly homologous genomes in polyploid and hemisexual dog roses, *Rosa* L. sect. *Caninae* DC. *Heredity* **92:** 139-150.

**Nybom H, Esselink GD, Werlemark G, Leus L, Vosman B (2006)** Unique genomic configuration revealed by microsatellite DNA in polyploid dog roses, *Rosa* sect. *Caninae*. *Journal of Evolutionary Biology* **19:** 635-648.

**Ode PJ (2006)** Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. *Annual Review of Entomology* **51:** 163-185.

**Ode PJ, Berenbaum MR, Zangerl AR, Hardy ICW (2004)** Host plant, host plant chemistry and the polyembryonic parasitoid *Copidosoma sosares*: indirect effects in a tritrophic interaction. *Oikos* **104:** 388-400.

**Orians CM (2000)** The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant-herbivoure interactions. *American Journal of Botany* **87:** 1749-1756.

**Orr DB, Boethel DJ (1986)** Influence of plant antibiosis through 4 trophic levels. *Oecologia* **70:** 242-249.

**Oshaghi MA, Yaaghoobi F, Abaie MR (2006)** Pattern of mitochondrial DNA variation between and within *Anopheles stephensi* (Diptera: Culicidae) biological forms suggests extensive gene flow. *Acta Tropica* **99**: 226-233.

**Oshaghi MA, Shemshad Kh, Yaghobi-Ershadi MR, Pedram M, Vatandoost H, Abaie MR, Akbarzadeh K, Mohtarami F (2007)** Genetic structure of the malaria vector *Anopheles superpictus* in Iran using mitochondrial cytochrome oxidase (COI and COII) and morphologic markers: A new species complex? *Acta Tropica* **101**: 241-248.

**Oshida T, Abramov A, Yanagawa H, Masuda R (2005)** Phylogeography of the Russian flying squirrel (*Pteromys volans*): implication of refugia theory in arboreal small mammal of Eurasia. *Molecular Ecology* **14:** 1191-1196.

**Pannebakker BA, Zwaan BJ, Beukeboom LW, Van Alphen JJM (2004)** Genetic diversity and *Wolbachia* infection of the *Drosophila* parasitoid *Leptopilina clavipes* in western Europe. *Molecular Ecology* **13:** 1119-1128.

**Peakall R, Smouse PE (2006)** GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6:** 288-295.

**Perkins SL (2001)** Phylogeography of Caribbean lizard malaria: tracing the history of vector-borne parasites. *Journal of Evolutionary Biology* **14:** 34-45.

**Pichersky E, Gang DR (2000)** Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends in Plant Science* **5:** 439-445.

**Pirozynski KA, Hawksworth, DL (1989)** Coevolution of fungi with plants and animals; introduction and overview. In: Pirozynski KA, Hawksworth DL (eds) Coevolution of fungi with plants and animals. Academic Press, London, pp 1-30.

**Plantard O, Rasplus JY, Mondor G, Le Clainche I, Solignac M (1998)** *Wolbachia*-induced thelytoky in the rose gallwasp *Diplolepis spinosissimae* (Giraud) (Hymenoptera: Cynipidae), and its consequences on the genetic structure of its host. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265:** 1075-1080.

**Porretta D, Canestrelli D, Bellini R, Celli G, Urbanelli S (2007)** Improving insect pest management through population genetic data: a case study of the mosquito *Ochlerotatus caspius* (Pallas). *Journal of Applied Ecology* **44**: 682-691.

**Posada D, Crandall KA (1998)** MODELTEST: testing the model of DNA substitution. *Bioinformatics.* **14:** 817-818.

**Pramual P, Kuvangkadilok C, Baimai V, Walton C (2005)** Phylogeography of the black fly *Simulium tani* (Diptera: Simuliidae) from Thailand as inferred from mtDNA sequences. *Molecular Ecology* **14**: 3989-4001.

**Prezler RW, Boecklen WJ (1994)** A three-trophic-level analysis of the effects of plant hybridization on a leaf-mining moth. *Oecologia* **100:** 66-73.

**Price PW, Clancy KM (1986a)** Interactions among three trophic levels: gall size and parasitoid attack. *Ecology* **67:** 1593-1600.

**Price PW, Clancy KM (1986b)** Multiple effects of precipitation on *Salix lasiolepis* and populations of the stem-galling sawfly, *Euura lasiolepis*. *Ecological Research* **1:** 1-14.

**Pritchard JK, Stephens M, Donnelly P (2000)** Inference of population structure using multilocus genotype data. *Genetics* **155:** 945-959.

**R Development Core Team (2006)** R: A language and environment for statistical computing.

**Randolph S (2005)** The natural history of the rose bedeguar gall and its insect community. British Plant Gall Society.

**Ranger C M, Hower AA (2002)** Glandular trichomes on perennial alfalfa affect host-selection behavior of *Empoasca fabae*. *Entomologia Experimentalis et Applicata* **105:** 71-81.

**Redfern M, Askew RR (1992)** Plant galls. The Richmond Publishing Co. Ltd. Slough.

**Reed LK, Nyboer M, Markow TA (2007)** Evolutionary relationships of *Drosophila mojavensis* geographic host races and their sister species *Drosophila arizonae*. *Molecular Ecology* **16**: 1007-1022.

**Reichert H (1998)** Beobachtungen und Versuche zur Fortpflanzung der Apfelrose, *Rosa villosa* L. (*R. pomifera* J. Herrmann). *Delattinia* **24:** 159-166.

**Riegler M, Stauffer C (2002)** *Wolbachia* infections and superinfections in cytoplasmically incompatible populations of the European cherry fruit fly *Rhagoletis cerasi* (Diptera, Tephritidae). *Molecular Ecology* **11**: 2425-2434.

**Ritz CM, Wissemann V (2003)** Male correlated non-matroclinal character inheritance in reciprocal hybrids of *Rosa* section *Caninae* (DC.) Ser. (Rosaceae). *Plant Systematics and Evolution* **241:** 213-221.

**Ritz C M, Maier WFA, Oberwinkler F, Wissemann V (2005a)** Different evolutionary histories of two *Phragmidium* species infecting the same dog rose hosts. *Mycological Research* **109:** 603-609.

**Ritz CM, Schmuths H, Wissemann V (2005b)** Evolution by reticulation: European dogroses originated by multiple hybridization across the genus *Rosa*. *Journal of Heredity* **96:** 4-14.

**Ritz CM, Wissemann V (submitted)** Natural bidirectional hybridisation between *R. rubiginosa* L. and *R. canina* L. (*Rosa* sect. *Caninae* (DC.) Ser.) is rare but may be associated with an increase of ploidy level.

**Roberts AV (1975)** The nature and taxonomic significance of the system of inheritance in *Rosa nanothamnus* (Rosaceae). *Botanical Journal of the Linnean Society* **71:** 59-66.

**Rokas A, Atkinson RJ, Brown GS, West SA, Stone GN (2001)** Understanding patterns of genetic diversity in the oak gallwasp *Biorhiza pallida*: demographic history or a *Wolbachia* selective sweep? *Heredity* **87:** 294-304.

**Rokas A, Nylander JAA, Ronquist F, Stone GN (2002)** A maximum-likelihood analysis of eight phylogenetic markers in gallwasps (Hymenoptera: Cynipidae): Implications for insect phylogenetic studies. *Molecular Phylogenetics and Evolution* **22:** 206-219.

**Rokas A, Atkinson RJ, Webster L, Csoka G, Stone GN (2003)** Out of Anatolia: longitudinal gradients in genetic diversity support an eastern origin for a circum-Mediterranean oak gallwasp *Andricus quercustozae*. *Molecular Ecology* **12**: 2153-2174.

**Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003)** DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics.* **19:** 2496-2497.

**Rudgers JA, Whitney KD (2006)** Interactions between insect herbivores and a plant architectural dimorphism. *Journal of Ecology* **94:** 1249-1260.

**Santucci F, Emerson BC, Hewitt GM (1998)** Mitochondrial DNA phylogeography of European hedgehogs. *Molecular Ecology* **7:** 1163-1172.

**Savelkoul PHM, Aarts HJM, de Haas J, Dijkshoorn L, Duim B, Otsen M, Rademaker JLW, Schouls L, Lenstra JA (1999)** Amplified-fragment length polymorphism analysis: the state of an art. *Journal of Clinical Microbiology* **37:** 3083-3091.

**Savile DBO (1979)** Fungi as aids in higher plant classification. *The Botanical Review* **45:** 377-503.

**Schilthuizen M, Stouthamer R (1997)** Horizontal transmission of parthenogenesis-inducing microbes in *Trichogramma* wasps. *Proceedings of the Royal Society of London Series B-Biological Sciences* **264:** 361-366.

**Schilthuizen M, Stouthamer R (1998)** Distribution of *Wolbachia* among the guild associated with the parthenogenetic gall wasp *Diplolepis rosae*. *Heredity* **81:** 270-274.

**Schlumprecht H (1989)** Dispersal of the thistle gallfly *Urophora cardui* and its endoparasitoid *Eurytomas serratulae* (Hymenoptera, Eurytomidae). *Ecological Entomology* **14:** 341-348.

**Schmitt T (2007)** Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Frontiers in Zoology* **4:** 11

**Schmitt T (2009)** Biogeographical and evolutionary importance of the European high mountain systems. *Frontiers in Zoology* **6:** 9

**Scholler M (1994)** Die Erysiphales, Pucciniales und Ustilaginales der Vorpommerschen Boddenlandschaft. *Regensburger Mykologische Schriften* **6:** 1-325.

**Schoonhoven LM, Jermy T, von Loon JJA (1998)** Insect-plant biology. From physiology to evolution. Chapmann & Hall, London.

**Schrey NM, Reeve JD, Anderson FE (2005)** Mitochondrial DNA analysis of the bark beetle predator *Thanasimus dubius* F. (Coleoptera: Cleridae) reveals regional genetic differentiation. *Molecular Ecology* **14**: 3317-3324.

**Schröder D (1967)** *Diplolepis* (=*Rhodites*) *rosae* (L.) (Hym.: Cynipidae) and a review of its parasite complex in Europe. *Technical Bulletin of the Commonwealth Institute of Biological Control* **9:** 93-131

**Seddon JM, Santucci F, Reeve N, Hewitt GM (2002)** Caucasus Mountains divide postulated postglacial colonization routes in the white-breasted hedgehog, *Erinaceus concolor*. *Journal of Evolutionary Biology* **15:** 463-467.

**Setamou M, Jiang NQ, Schulthess F (2005)** Effect of the host plant on the survivorship of parasitized *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) larvae and performance of its larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). *Biological Control* **32:** 183-190.

**Sezonlin M, Dupas S, Le Ru B, Le Gall P, Moyal P, Calatayud PA, Giffard I, Faure N, Silvain JF (2006)** Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (Lepidoptera, Noctuidae) in sub-Saharan Africa. *Molecular Ecology* **15**: 407-420.

**Sha Z-L, Zhu C-D, Murphy RW, Salle JL, Huang D-W (2006)** Mitochondrial phylogeography of a leafminer parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae) in China. *Biological Control* **38**: 380-389.

**Shi W, Kerdelhue C, Ye H (2005)** Population genetics of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae), in Yunnan (China) based on mitochondrial DNA sequences. *Environmental Entomology* **34**: 977-983.

**Shorthouse JD, Leggo JJ, Sliva MD, Lalonde RG (2005a)** Has egg location influenced the radiation of *Diplolepis* (Hymenoptera: Cynipidae) gall wasps on wild roses? *Basic and Applied Ecology* **6:** 423-434.

**Shorthouse JD, Wool D, Raman A (2005b)** Gall-inducing insects – Nature's most sophisticated herbivores. *Basic and Applied Ecology* **6:** 407-411.

**Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach AT (2006)** Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics* **37:** 545-579.

**Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994)** Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**: 651-701.

**Simonato M, Mendel Z, Kerdelhue C, Rousselet J, Magnoux E, Salvato P, Roques A, Battisti A, Zane L (2007)** Phylogeography of the pine processionary moth *Thaumetopoea wilkinsoni* in the Near East. *Molecular Ecology* **16**: 2273-2283.

**Smith CI, Farrell BD (2005)** Phylogeography of the longhorn cactus beetle *Moneilema appressum* LeConte (Coleoptera: Cerambycidae): was the differentiation of the Madrean sky islands driven by Pleistocene climate changes? *Molecular Ecology* **14**: 3049-3065.

**Smith JJ, Bush GL (1997)** Phylogeny of the genus *Rhagoletis* (Diptera: Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxidase II. *Molecular Phylogenetics and Evolution* **7**: 33-43.

**Smith JJ, Bush GL (2000)** Phylogeny of the subtribe Carpomyina (Trypetinae), emphasizing relationships of the genus *Rhagoletis*. In Aluja M. & Norrbom A.L. (eds): *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior*. CRC Press, Boca Raton, FL, pp. 187-217.

**Smith P (2005)** Mitochondrial DNA variation among populations of the glassy-winged sharpshooter, *Homalodisca coagulate*. *Journal of Insect Science* **5.41**: 1-8.

**Smouse PE, Peakall R (1999)** Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity* **82:** 561-573.

**Snäll N, Huoponen K, Saloniem I, Savontaus M-L, Ruohomäki K (2004)** Dispersal of females and differentiation between populations of *Epirrita autumnata* (Lepidoptera: Geometridae) inferred from variation in mitochondrial DNA. *European Journal of Entomology* **101**: 495-502.

**Sperling FAH, Raske AG, Otvos IS (1999)** Mitochondrial DNA sequence variation among populations and host races of *Lambdina fiscellaria* (Gn.) (Lepidoptera: Geometridae). *Insect Molecular Biology* **8:** 97-106.

**Stauffer C, Lakatos F, Hewitt GM (1999)** Phylogeography and postglacial colonization routes of *Ips typographus* L-(Coleoptera, Scolytidae). *Molecular Ecology* **8:** 763-773.

**Stechmann D-H, Bauer G, Dreyer W, Heusinger G, Zwölfer H (1981)** Die Erfassung der Entomofauna von Heckenpflanzen (Wildrose, Schlehe, Weißdorn) mit Hilfe der Klopfprobenmethode. *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie*. **3:** 12-26.

**Stiling P, Rossi AM (1997)** Experimental manipulations of top-down and bottom-up factors in a tri-trophic system. *Ecology* **78:** 1602-1606.

**Stille B (1984)** The effect of host plant and parasitoids on the reproductive success of the parthenogenetic gall wasp *Diplolepis rosae* (Hymenoptera, Cynipidae). *Oecologia* **63:** 364-369.

**Stille B, Dävring L (1980)** Meiosis and reproductive strategy in the parthenogenetic gall wasp *Diplolepis rosae* (L.) (Hymenoptera, Cynipidae). *Hereditas* **92:** 353-362.

**Stone G, Atkinson R, Rokas A, Csoka G, Nieves-Aldrey JL (2001)** Differential success in northwards range expansion between ecotypes of the marble gallwasp *Andricus kollari*: a tale of two lifecycles. *Molecular Ecology* **10:** 761-778.

**Stone GN, Schönrogge K, Atkinson RJ, Belldio D, Pujade-Villar J (2002)** The population biology of oak gall wasps (Hymenoptera: Cynipidae). *Annual Review of Entomology* **47:** 633-668.

**Stone G, Schönrogge K (2003)** The adaptive significance of insect gall morphology. *Trends in Ecology & Evolution* **18:** 512-522.

**Stone GN, Hernandez-Lopez A, Nicholls JA, di Pierro E, Pujade-Villar J, Melika G, Cook JM (2009)** Extreme host plant conservatism during at least 20 million years of host plant pursuit by oak gallwasps. *Evolution* **63:** 854-869.

**Stouthamer R, Breeuwer JAJ, Luck RF, Werren JH (1993)** Molecular identification of microorganisms associated with parthenogenesis. *Nature* **361:** 66-68.

**Stouthamer R, Breeuwer JAJ, Hurst GDD (1999)** *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. *Annual Review of Microbiology* **53:** 71-102.

**Strong DR, Lawton JH, Southwood R (1984)** Insects on plants. Community patterns and mechanisms. Blackwell Scientific Publications, Oxford.

**Swofford DL (2002)** Paup: Phylogenetic analysis using parsimony (and other methods) 4.0 beta. *Sunderland MA: Sinauer Associates*

**Sword GA, Joern A, Senior LB (2005)** Host plant-associated genetic differentiation in the snakeweed grasshopper, *Hesperotettix viridis* (Orthoptera: Acrididae). *Molecular Ecology* **14:** 2197-2205.

**Szalanski AL, Owens CB, Lewter JA, Borce AB (2006)** Genetic structure of *Aedes vexans* (Diptera: Culicidae) populations from Central United States based on mitochondrial ND5 sequences. *Annals of the Entomological Society of America* **99**: 157-163.

**Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF (1998)** Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* **7:** 453-464.

**Täckholm G (1920)** On the cytology of the genus *Rosa*. A preliminary note. *Svensk Bot Tidskr* **14:** 300-311.

**Täckholm G (1922)** Zytologische Studien über die Gattung *Rosa*. *Acta Hort Bergianai* **7.3:** 97-381.

**Themu EA, Yan G (2005)** Microsatellite and mitochondrial genetic differentiation of *Anopheles arabiensis* (Diptera: Culicidae) from western Kenya, the great rift valley and coastal Kenya. *The American Society of Tropical Medicine and Hygiene* **73**: 726-733.

**Thompson JN (2005)** The geographic mosaic of coevolution. University of Chicago Press. Chicago, IL.

**Thompson AR, Thacker CE, Shaw EY (2005)** Phylogeography of marine mutualists: parallel patterns of genetic structure between obligate goby and shrimp partners. *Molecular Ecology* **14:** 3557-3572.

**Timmermann G. (1998)** Beobachtungen zur Phänologie der heimischen Wildrosen in Rottenburg am Neckar. *Acta Rhodologica* **1:** 7-14.

**Timmermann G, Müller T (1994)** Wildrosen und Weißdorne Mitteleuropas – Landschaftsgerechte Sträucher und Bäume. Verlag des Schwäbischen Albvereins e. V., Stuttgart.

**Timmermans MJTN, Ellers J, Marien J, Verhoef SC, Ferwerda EB, Van Straalen NM (2005)** Genetic structure in *Orchesella cincta* (Collembola): strong subdivision of European populations inferred from mtDNA and AFLP markers. *Molecular Ecology* **14**: 2017-2024.

**Tscharntke T, Greiler HJ (1995)** Insect communities, grasses, and grasslands. **Annual Review of Entomology 40:** 535-558.

**Tscharntke T, Vidal S, Hawkins BA (2001)** Parasitoids of grass-feeding chalcid wasps: a comparison of German and British communities. *Oecologia* **129:** 445-451.

**Valkama E, Koricheva J, Salminen J.-P, Helander M, Saloniemi I, Saikkonen K, Pihlaja K (2005)** Leaf surface traits: overlooked determinants of birch resistance to herbivores and foliar micro-fungi? *Trees* **19:** 191-197.

**Vandewoestijne S, Baguette M, Brakefield PM, Saccheri IJ (2004)** Phylogeography of *Aglais urticae* (Lepidoptera) based on DNA sequences of the mitochondrial COI gene and control region. *Molecular Phylogenetics and Evolution* **31**: 630-646.

**Vasconcelos T, Horn A, Lieutier F, Branco M, Kerdelhue C (2006)** Distribution and population genetic structure of the Mediterranean pine shoot beetle *Tomicus destruens* in the Iberian Peninsula and Southern France. *Agricultural and Forest Entomology* **8**: 103-111.

**Vaupel A, Klinge K, Brändle M, Wissemann V, Tscharntke T, Brandl R (2007)** Genetic differentiation between populations of the European rose hip fly *Rhagoletis alternata*. *Biological Journal of the Linnean Society* **90:** 619-625.

**Vila M, Björklund M (2004a)** Testing biennialism in the butterfly *Erebia palarica* (Nymphalidae: Satyrinae) by mtDNA sequencing. *Insect Molecular Biology* **13**: 213-217.

**Vila M, Björklund M (2004b)** The utility of the neglected mitochondrial control region for evolutionary studies in Lepidoptera (Insecta). *Journal of Molecular Evolution* **58**: 280-290.

**Vila M, Vidal-Romani JR, Björklund M (2005)** The importance of time scale and multiple refugia: Incipient speciation and admixture of lineages in the butterfly *Erebia triaria* (Nymphalidae) *Molecular Phylogenetics and Evolution* **36**: 249-260.

**Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995)** AFLP - a new technique for DNA fingerprinting. *Nucleic Acids Research* **23:** 4407-4414.

**Wagener B, Reineke A, Lohr B, Zebitz CPW (2006)** Phylogenetic study of *Diadegma* species (Hymenoptera: Ichneumonidae) inferred from analysis of mitochondrial and nuclear DNA sequences. *Biological Control* **37:** 131-140.

**Wagner WL, Funk VA (1995)** Hawaiian biogeography: Evolution on a hot spot archipelago. Washington, DC: Smithsonian Institution Press.

**Weir BS, Cockerham CC (1984)** Estimating *F*-statistics for the analysis of population structure. *Evolution* **38:** 1358-1370.

**Weis AE (1983)** Patterns of parasitism by *Torymus capite* on hosts distributed in small patches. *Journal of Animal Ecology* **52:** 867-877.

**Weis AE, Abrahamson WG (1985)** Potential selective pressure by parasitoids on a plant-herbivore interaction. *Ecology* **66:** 1261-1269.

**Werlemark G, Uggla M, Nybom H (1999)** Morphological and RAPD markers show a highly skewed distribution in a pair of reciprocal crosses between hemisexual dog rose species, *Rosa* sect. *Caninae*. *Theoretial and Applied Genetics* **98:** 557-563.

**Werlemark G, Nybom H (2001)** Skewed distribution of morphological character scores and molecular markers in three interspecific crosses in *Rosa* sect. *Caninae*. *Hereditas* **134:** 1-13.

**Werren JH (1997)** Biology of *Wolbachia*. *Annual Review of Entomology* **42:** 587-609.

**Werren JH, Baldo L, Clark ME (2008)** *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews Microbiology* **6:** 741-751.

**White I (1988)** Tephritid flies (Handbooks for the identification of British insects 10, part 5a). Royal Entomological Society, London.

**White IM, Elson-Harris MM (1992)** *Fruit flies of economic significance: Their identification and bionomics*. CABI Publishing, Wallingford, Oxon, UK, pp. 601.

**White TJ, Bruns T, Lee S, Taylor J (1990)** Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A guide to methods and applications. Academic Press, Inc. 315-322.

**Whitham TG, Young WP, Martinsen GD, Gehring CA, Schweitzer JA, Shuster SM, Wimp GM, Fischer DG, Bailey JK, Lindroth RL, Woolbright S, Kuske CR (2003)** Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology* **84:** 559-573.

**Williams HC, Ormerod SJ, Bruford MW (2006)** Molecular systematics and phylogeography of the cryptic species complex *Baetis rhodani* (Ephemeroptera, Baetidae). *Molecular Phylogenetics and Evolution* **40**: 370-382.

**Wink M, Grimm C, Koschmieder C, Sporer F, Bergeot O (2000)** Sequestration of phorbolesters by the aposematically coloured bug *Pachycoris klugii* (Heteroptera: Scutelleridae) feeding on *Jatropha curcas* (Euphorbiaceae). *Chemoecology* **10:** 179-184.

**Wissemann V (1999)** Genetic constitution of *Rosa* sect. *Caninae* (*R. canina, R. jundzillii*) and sect. *Gallicanae* (*R. gallica*). *Angewandte Botanik* **73:** 191-196.

**Wissemann V (2000a)** Epicuticular wax morphology and the taxonomy of *Rosa* (section *Caninae,* subsection *Rubiginosae*) *Plant Systematics and Evolution* **221:** 107-112.

**Wissemann V (2000b)** Molekulargenetische und morphologisch-anatomische Untersuchun-gen zur Evolution und Genomzusammensetzung von Wildrosen der Sektion *Caninae* (DC.) Ser. *Botanische Jahrbücher für Systematik* **122.3:** 357-429.

**Wissemann V (2002)** Molecular evidence for allopolyploid origin of the *Rosa canina*-complex (Rosaceae, Rosoideae). *Journal of Applied Botany* **76**: 176-178.

**Wissemann V (2005)** Evolution by hybridisation. The influence of reticulate evolution on biosymmetrical patterns and processes in plants. *Theory in Biosciences* **123:** 223-233.

**Wissemann V, Hellwig FH (1997)** Reproduction and hybridisation in the genus *Rosa*, section *Caninae* (Ser.) *Rehd Bot Acta* **110:** 251-256.

**Wissemann, V, Ritz, C (2005)** The genus *Rosa* (Rosoideae, Rosaceae) revisited: molecular analysis of nrITS-1 and atpB-rbcL intergenic spacer (IGS) versus conventional taxonomy. *Botanical Journal of the Linnean Society* **147**: 275-290.

**Wissemann V, Gallenmüller F, Ritz C, Steinbrecher T, Speck T (2006)** Inheritance of growth form and mechanical characters in reciprocal polyploid hybrids of *Rosa* section *Caninae* - implications for the ecological niche differentiation and radiation process of hybrid offspring. *Trees-Structure and Function* **20:** 340-347.

**Wissemann, V, Ritz, C (2007)** Evolutionary patterns and processes in the genus *Rosa* (Rosaceae) and their implications for host-parasite co-evolution. *Plant Systematics and Evolution* **266:** 79-90.

**Wissemann V, Riedel M, Riederer M (2007)** Matroclinal inheritance of cuticular waxes in reciprocal hybrids of *Rosa* sect. *Caninae* (Rosaceae). *Plant Systematics and Evolution* **263:** 181-190.

**Yencho G C, Tingey W M (1994)** Glandular trichomes of *Solanum berthaultii* alter host preference of the colorado potatoe beetle, *Leptinotarsa decemlineata*. *Entomologia Experimentalis et Applicata* **70:** 217-225.

**Zenger KR, Eldridge MDB, Johnston PG (2005)** Phylogenetics, population structure and genetic diversity of the endangered southern brown bandicoot (*Isoodon obesulus*) in south-eastern Australia. *Conservation Genetics* **6:** 193-204.

**Zielinski J (1985)** Studia nad rodzajem Rosa L. - Systematyka sekcji *Caninae* DC. em. Christ. *Arboretum Kornickie* **30:** 3-109.

**Zvereva E L, Kozlov MV, Niemelä P (1998)** Effects of leaf pubescence in *Salix borealis* on host-plant choice and feeding behavior of the leaf beetle, *Melanosoma lapponica*. *Entomologia Experimentalis et Applicata* **89:** 297-303.

**Zwölfer H, Bauer G, Heusinger G (1981)** Ökologische Funktionsanalyse von Feldhecken – Tierökologische Untersuchungen über Struktur und Funktion biozönotischer Komplexe. Schlußbericht an das Bayerische Landesamt für Umweltschutz, München.

**Zwölfer H, Bauer G, Heusinger G, Stechmann D (1984)** Die tierökologische Bedeutung und Bewertung von Hecken. Beiheft 3, Teil 2, zu den Berichten der Akademie für Naturschutz und Landschaftspflege, Laufen.

# 9. Appendix

Table A1. Localities at which *Rhagoletis alternata* and *Carpomya schineri* were sampled in Europe.

| Species | Number of specimens | Country | Locality | Lat. | Long. | Collector |
|---|---|---|---|---|---|---|
| *Rh. alternata* | 2 | Austria | Seesattel | 46.4261 | 14.5305 | H. Rieger-Hager |
| | 1 | Austria | Corte | 46.5333 | 12.1333 | M. Brändle |
| | 2 | France | St. Bernhard | 45.6278 | 6.8304 | M. Brändle |
| | 2 | Germany | Oldenburg | 53.0000 | 8.0000 | Botanical Garden |
| | 2 | Germany | Marburg | 50.8167 | 8.7667 | A. Kohnen |
| | 2 | Germany | Augsburg | 48.3500 | 10.9000 | Botanical Garden |
| | 1 | Germany | Greifswald | 54.0833 | 13.3833 | S. Starke |
| | 2 | Germany | Kiel | 54.3333 | 10.1333 | A. Kohnen |
| | 2 | Italy | Aosta | 45.8992 | 7.4839 | M. Brändle |
| | 1 | Netherlands | De Koog | 53.0833 | 4.7500 | W. Koopman |
| | 1 | Slovakia | Martin | 49.0189 | 18.7891 | M. Brändle |
| | 3 | Switzerland | Ulrichen | 46.3044 | 8.1778 | A. Vaupel |
| *C.schineri* | 4 | Austria | Vienna | 48.2167 | 16.3667 | F. Tod |
| | 1 | Austria | Sandeck | 47.8333 | 16.7500 | R. Brandl |
| | 2 | Germany | Berlin | 52.5333 | 13.4167 | M. Raddatz |
| | 3 | Italy | Siena | 43.3167 | 11.3167 | P. Castagnini |
| | 3 | Spain | Jaca | 42.5667 | -0.5500 | H. Zimmermann |

Table A2. Studies on the number of haplotypes in insects used in the comparison with those found in this study on *Rhagoletis alternata* and *Carpomya schineri*.

| Species | Locus | Length (bp) | No. of specimen sequenced | No. of haplo-types | Reference |
|---|---|---|---|---|---|
| *Aedes aegypti* | NADH 4 | 361 | 55 | 3 | da Costa-da-Silva et al. 2005 |
| *Aedes vexans* | NADH 5 | 423 | 54 | 34 | Szalanski et al. 2006 |
| *Agathis n. sp.* | CO I - CO II | 1260 | 78 | 42 | Althoff et al. 2001 |
| *Aglais urticae* | CO I | 1216 | 67 | 44 | Vandewoestijne et al. 2004 |
| *Aglais urticae* | D-loop | 715 | 62 | 22 | Vandewoestijne et al. 2004 |
| *Andricus quercustozae* | Cytb | 433 | 45 | 25 | Rokas et al. 2003 |
| *Anopheles albimanus* | Cytb | 222 | 7 | 5 | Airas et al. 2005 |
| *Anopheles arabiensis* | NADH 5 | 595 | 196 | 39 | Themu et al. 2005 |
| *Anopheles flavirostris* | CO I | 261 | 102 | 16 | Foley et al. 2006 |
| *Anopheles stephensi* | CO I | 877 | 4 | 1 | Oshaghi et al. 2006 |
| *Anopheles stephensi* | CO II | 640 | 12 | 2 | Oshaghi et al. 2006 |
| *Anopheles superpictus* | CO I | 708 | 18 | 8 | Oshaghi et al. 2007 |
| *Aphidus ervi* | CO I - CO II | 2000 | 78 | 12 | Hufbauer et al. 2004 |
| *Bactrocera depressa* | CO I | 821 | 63 | 20 | Mun et al. 2003 |
| *Bactrocera dorsalis* | CO I | 505 | 37 | 28 | Shi et al. 2005 |
| *Bactrocera oleae* | NADH 1 | 574 | 93 | 22 | Nardi et al. 2005 |
| *Baetis rhodani* | CO I | 472 | 360 | 68 | Williams et al. 2006 |
| *Busseola fusca* | Cytb | 965 | 307 | 70 | Sezonlin et al. 2006 |
| *Carpomya schineri* | COI, COII, Cyt b | 1720 | 10 | 1 | Present study |
| *Chorthippus parallelus* | CO I | 300 | 90 | 31 | Lunt et al. 1998 |
| *Culicoides imicola* | CO I | 472 | 50 | 11 | Dallas et al. 2003 |
| *Dendroctonus rufipennis* | CO I | 1114 | 93 | 66 | Maroja et al. 2007 |
| *Diglyphus isaea* | CO I | 745 | 29 | 28 | Sha et al. 2006 |
| *Drosophila arizonae* | CO I | 658 | 100 | 41 | Reed et al. 2007 |
| *Drosophila lacertosa* | NADH 2 | 1026 | 213 | 71 | He et al. 2007 |
| *Drosophila mojavensis* | CO I | 658 | 174 | 46 | Reed et al. 2007 |
| *Drosophila montanta* | CO I + CO II | 1358 | 108 | 72 | Mirol et al. 2007 |
| *Epirrita autumnata* | D-loop | 542 | 200 | 108 | Snall et al. 2004 |
| *Erebia palarica* | CO I | 786 | 60 | 5 | Vila et al. 2004b |
| *Erebia palarica* | D-loop | 354 | 60 | 7 | Vila et al. 2004b |
| *Erebia palarica* | D-Loop + CO I | 1142 | 40 | 16 | Vila et al. 2004a |
| *Erebia triaria* | CO I | 786 | 60 | 14 | Vila et al.2005 |
| *Erebia triaria* | CO I - CO II | 1147 | 126 | 46 | Vila et al. 2004b |
| *Erebia triaria* | D-loop | 361 | 60 | 10 | Vila et al. 2004b |
| *Gonioctena olivacea* | CO I | 1176 | 58 | 6 | Mardulyn et al. 2005 |
| *Gonioctena olivacea* | D-loop 1 | 1875 | 116 | 48 | Mardulyn et al. 2005 |
| *Gonioctena olivacea* | D-loop 2 | 649 | 191 | 32 | Mardulyn et al. 2005 |
| *Homalodisca coagulata* | CO I | 486 | 57 | 14 | Smith 2005 |
| *Hyalopterus* | CO I | 343 | 155 | 6 | Lozier et al. 2007 |
| *Hyles tithymali* | CO I - CO II | 2295 | 43 | 18 | Hundsdoerfer et al. 2006 |
| *Ips typographus* | CO I | 902 | 136 | 8 | Stauffer et al. 1999 |
| *Moneilema appressum* | CO I | 1413 | 64 | 63 | Smith et al. 2005 |
| *Ochlerotatus caspius* | CO II | 526 | 173 | 21 | Porretta et al. 2007 |
| *Orchesella cincta* | CO II | 563 | 52 | 16 | Timmermans et al. 2005 |
| *Ostrinia nubilalis* | CO I - CO II | 2156 | 14 | 14 | Coates et al. 2004 |

Table A2. continued.

| Species | Locus | Length (bp) | No. of specimen sequenced | No. of haplo-types | Reference |
|---|---|---|---|---|---|
| *Phlebotomus papatasi* | Cytb - NADH1 | 441 | 27 | 16 | Esseghir et al. 1997 |
| *Pissodes strobi* | CO I | 826 | 130 | 36 | Laffin et al. 2004 |
| *Plutella xylostella* | CO I | 681 | 80 | 16 | Li et al. 2006 |
| *Polyura eudamippus* | CO II | 405 | 50 | 9 | Long et al. 2006 |
| *Rhagoletis alternata* | COI, COII, Cyt b | 1720 | 10 | 1 | Present study |
| *Simulium tani* | CO I | 720 | 147 | 121 | Pramual et al. 2005 |
| *Spodoptera frugiperda* | CO I - CO II | 608 | 71 | 7 | Lewter et al. 2006 |
| *Synemon plana* | CO II | 715 | 230 | 14 | Clarke et al. 2003 |
| *Tarphius canariensis* | CO I + CO II | 1041 | 87 | 63 | Emerson et al. 2000 |
| *Tegeticula yuccasella* | CO I | 844 | 170 | 51 | Leebens-Mack et al. 2004 |
| *Tephritis bardanae* | CO I - CO II | 830 | 140 | 18 | Diegisser et al. 2004 |
| *Thanasimus dubius* | CO I | 464 | 85 | 60 | Schrey et al. 2005 |
| *Thaumetopoea wilkinsoni* | CO I | 262 | 192 | 11 | Simonato et al. 2007 |
| *Thaumetopoea wilkinsoni* | CO II | 342 | 192 | 15 | Simonato et al. 2007 |
| *Tomicus destruens* | CO I - CO II | 617 | 113 | 26 | Vasconcelos et al. 2006 |
| *Trypocopris alpinus* | CO I | 568 | 10 | 9 | Carisio et al. 2004 |
| *Trypocopris pyrenaeus* | CO I | 568 | 25 | 16 | Carisio et al. 2004 |
| *Trypocopris vernalis* | CO I | 568 | 14 | 13 | Carisio et al. 2004 |

Table A3. Sample locality of *Diplolepis rosae* and its two parasitoid species *Orthopelma mediator* and *Glyphomerus stigma* in Euorpe. The number of individuals (Ind.), the haplotype of COI (COI) and ITS 2 (ITS 2) sequences, and the presence (+) or absence (-) of *Wolbachia* bacteria (*Wolb.*) are recorded, respectively.

| Country | Location | *D. rosae* | | | *O. mediator* | | | | *G. stigma* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ind. | COI | *Wolb.* | Ind. | COI | ITS 2 | *Wolb.* | Ind. | COI | *Wolb.* |
| Austria | Sandeck | 3 | Hap3 | +++ | 3 | Hap1 | Hap2 | - - + | 1 | Hap1 | - |
| | Klagenfurt | 1 | Hap2 | + | 0 | | | | 0 | | |
| | Koralpe | 1 | Hap1 | + | 0 | | | | 0 | | |
| Czech | Průhonice | 1 | Hap1 | + | 1 | Hap1 | Hap1 | - | 0 | | |
| | Olomouc | 2 | Hap1 | ++ | 1 | Hap7 | Hap3 | - | 1 | Hap16 | - |
| Denmark | Tversted | 3 | Hap8 | +++ | 3 | Hap12; 13; | Hap7 | +++ | 0 | | |
| | Horsholm | 3 | Hap1 | +++ | 3 | Hap11 | Hap7 | - - - | 0 | | |
| Finland | Örö | 3 | Hap1 | +++ | 2 | Hap2 | Hap6 | - - | 0 | | |
| France | Nantes | 3 | Hap13 | +++ | 3 | Hap18; 19 | Hap5 | +++ | 2 | Hap19 | - - |
| | Chelles | 3 | Hap7 | - - + | 3 | Hap8; 20 | Hap4 | +++ | 0 | | |
| | Sarlat-et-Caneda | 3 | Hap1 | +++ | 2 | Hap21; 22 | Hap5 | + - | 0 | | |
| | Chateau-Challon | 3 | Hap1; 4; | +++ | 1 | Hap23 | Hap8 | - | 0 | | |
| | Perigueux | 2 | Hap1 | + - | 0 | | | | 0 | | |
| | Doucier | 0 | | | 0 | | | | 2 | Hap20; 21 | - - |
| Germany | Ulm | 3 | Hap4 | +++ | 1 | Hap2 | Hap2 | + | 0 | | |
| | Darmstadt | 2 | Hap7 | ++ | 2 | Hap1; 2 | Hap3 | + - | 0 | | |
| | Salzgitter | 3 | Hap1; 4 | +++ | 3 | Hap1; 2 | Hap3 | - - - | 3 | Hap5; 6 | - - - |
| | Sellin | 1 | Hap1 | + | 1 | Hap8 | Hap3 | - | 0 | | |
| | Poel | 1 | Hap1 | + | 2 | Hap2 | Hap3 | - - | 0 | | |
| Hungary | Matrafured | 1 | Hap4 | + | 1 | Hap9 | Hap6 | - | 3 | Hap8; 10; 11 | - - - |

Table A3. continued.

| Country | Location | *D. rosae* | | | *O. mediator* | | | | *G. stigma* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ind. | COI | *Wolb.* | Ind. | COI | ITS 2 | *Wolb.* | Ind. | COI | *Wolb.* |
| Italy | Monte Baldo | 2 | Hap1 | ++ | 2 | Hap2; 3 | Hap3 | - - | 0 | | |
| | Siena | 3 | Hap5; 6 | + - - | 1 | Hap4 | Hap4 | | 3 | Hap2; 3; 4 | - - - |
| Lithuania | Kaunas | 0 | | | 3 | Hap1; 2 | | | 0 | | |
| Macedonia | Ohrid | 2 | Hap14; 15 | + - | 1 | Hap2 | Hap3 | + | 2 | Hap23; 24 | - - |
| Netherlands | Haaren | 1 | Hap1 | + | 2 | Hap2 | Hap6 | - - | 0 | | |
| | Meeden | 1 | Hap9 | - | 0 | | | | 0 | | |
| | Haren | 1 | Hap1 | - | 0 | | | | 0 | | |
| | Rhenen | 1 | Hap1 | - | 0 | | | | 0 | | |
| Norway | Lier | 3 | Hap1 | - ? - | 0 | | | | 0 | | |
| Poland | Baligrod | 1 | Hap1 | + | 0 | | | | 0 | | |
| Slovakia | Martin | 3 | Hap4 | +++ | 3 | Hap1; 6; 7 | Hap3 | - - - | 3 | Hap7; 8; 9 | - - - |
| Spain | Leon | 3 | Hap11 | +++ | 3 | Hap10; 16 | Hap5 | - - + | 3 | Hap13; 14; 15 | - - - |
| | Huesca | 3 | Hap12 | +++ | 2 | Hap11; 17 | Hap5 | - - | 2 | Hap17; 18 | - - - |
| | Burgos | 1 | Hap1 | + | 0 | | | | 2 | Hap12; 13 | - - |
| Sweden | Stockholm | 1 | Hap1 | + | 1 | Hap11 | Hap5 | - | 0 | | |
| | Uppsala | 3 | Hap1 | +++ | 2 | Hap11; 12 | Hap5 | - - | 0 | | |
| UK | Low | 3 | Hap8 | +++ | 1 | Hap5 | Hap5 | - | 0 | | |
| | Langford | 2 | Hap1; | ++ | 3 | Hap11; 15; | Hap5 | +++ | 0 | | |
| Ukraine | Kiew | 3 | Hap4 | +++ | 0 | | | | 1 | Hap22 | - |

## Erklärung zu eigenen Beiträgen und Veröffentlichungen

**Kapitel 2**: Radiation, biological diversity and host-parasite interactions in wildroses, rust fungi and insects

Aus “Evolution in Action” eds. Glaubrecht, M., Schneider, H., (2009), Chapter: Radiation, biological diversity and host-parasite interactions in wildroses, rust fungi and insects

Das Buch “Evolution in Action” fasst die Ergebnisse des Schwerpunktprogramms SPP 1127 „Radiationen - Genese biologischer Vielfalt“ der DFG zusammen. Jedes Projekt gestaltete ein Kapitel. Das hier verwendete Kapitel entstand aus dem Projekt „Radiation, Biodiversität und Wirt-Parasit Interaktionen im Rosen-System“ in dessen Rahmen ich meine Doktorarbeit anfertigte. Es fasst die Ergebnisse aller beteiligten Arbeitsgruppen zu einem Review zusammen. Das Kapitel wurde zu gleichen Teilen von Prof. Volker Wissemann und mir geschrieben. Im Unterkapitel 6 wurden von mir Ergebnisse zweier Diplomarbeiten (von Andrea Vaupel und Roman Fricke), einer Doktorarbeit (von Katrin Klinge) und Teile meiner eigenen Ergebnisse verwendet.

**Kapitel 3**: Cynipid gall-parasitoid interactions, comparing three dog rose species along a geographical gradient

*Annette Kohnen, Katrin Klinge, Volker Wissemann, Teja Tscharntke, Roland Brandl*

Prof. Volker Wissemann, Prof. Roland Brandl und Prof. Teja Tscharntke waren an der Planung der Untersuchung beteiligt. Der Datensatz wurden von Katrin Klinge im Rahmen ihrer Doktorarbeit aufgenommen, von mir jedoch neu zusammengestellt und ausgewertet. Das Manuskript wurde im Wesentlichen von mir verfasst. In der Auswertung unterstützt wurde ich von Prof. Roland Brandl, der außerdem das Manuskript korrigiert und verbessert hat.

**Kapitel 4**: No host-associated differentiation in the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae) on three dog rose species

*Annette Kohnen, Volker Wissemann, Roland Brandl*

Die Rosengallen wurden im Wesentlichen von mir, an manchen Standorten mit Hilfe von Prof. Volker Wissemann und Dr. Christiane Ritz gesammelt. Dabei wurden die Rosenarten von Prof. Volker Wissemann bestimmt oder gegebenenfalls nachbestimmt und korrigiert. Beim Aussortieren der geschlüpften Insektenarten haben mir zwei Hilfskräfte Sandra Schneider und Antje Schmidt geholfen. Die Laborarbeit und die Auswertung wurden von mir ausgeführt. Auch das Manuskript wurde im Wesentlichen von mir verfasst und von Prof. Roland Brandl korrigiert. Es wurde in ähnlicher Form bei dem Journal „*Biological Journal of the Linnean Society*“ als Artikel eingereicht.

**Kapitel 5**: Comparing geographical structures of one cynipid gall wasp with two specialised parasitoids in Europe

*Annette Kohnen, Iris Richter, Volker Wissemann, Roland Brandl*

Auf Anfragen wurde mir das Probenmaterial, die Rosengallen, von Botanischen Gärten, Universitäten und Privatpersonen zugestellt. Die gesammelten Gallen wurden in Plastikboxen im Freien gelagert, so dass die Insekten schlüpfen konnten. Bei der Aussortierung und Bestimmung der Insekten haben mir zwei Hilfskräfte Sandra Schneider und Antje Schmidt geholfen. Die Laborarbeit wurde zum Teil von Iris Richter im Rahmen ihrer Bachlorarbeit unter meiner Anleitung durchgeführt. Zur Erweiterung des Datensatzes wurde die Laborarbeit von mir selber durchgeführt. Der gemeinsame Datensatz wurde von mir ausgewertet und zu einem Manuskript verarbeitet. Prof. Roland Brandl hat mich in der Auswertung und der Arbeit am Manuskript korrigiert.

**Kapitel 6**: No genetic differentiation in rose infesting fruit flies (*Rhagoletis alternata* Fall. and *Carpomya schineri* Loew) across Central Europe.

*Annette Kohnen, Volker Wissemann, Roland Brandl* (2009). *European Journal of Entomology*, **106** (2), 315-321

Die Proben wurden mir nach Anfrage freundlicherweise von Botansichen Gärten und Wissenschaftlern zur Verfügung gestellt. Die Laborarbeit und die Auswertung wurden nach Absprache mit Prof. Roland Brandl von mir vorgenommen. Das Manuskript wurde im Wesentlichen von mir in Zusammenarbeit mit Prof. Volker Wissemann und Prof. Roland Brandl verfasst.

## Erklärung

Ich versichere, dass ich meine Dissertation „**Radiation, biological diversity and host-parasite interactions in wild roses and insects**“ selbständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe. Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Marburg, Oktober 2009

*„Mühsam ernährt sich das Eichhörnchen“*

Zum Glück fand es Hilfe in der Not bei vielen Freunden und Kollegen. An dieser Stelle möchte ich mich bei all jenen bedanken, die mich in den letzten 3 ½ Jahren während der Arbeit an meiner Dissertation unterstützt haben.

Mein besonderer Dank gilt Roland Brandl für die Betreuung meiner Arbeit und seine Unterstützung insbesondere in allen Statistikfragen.

Ebenfalls bedanken möchte ich mich bei Volker Wissemann. Seine Rosenkenntnisse sowohl der lebenden Pflanze als auch der Literatur und seine stetige Hilfsbereitschaft haben mir sehr geholfen.

Außerdem möchte ich mich hier bei den Mitgliedern der Prüfungskommission Lothar Beck und Nina Farwig bedanken, dass sie die Zeit gefunden haben, sich mit meiner Arbeit zu beschäftigen.

Eine Arbeit ohne Probenmaterial ist genauso undenkbar wie das Beschaffen des Materials durch eine einzige Person alleine. Auf meinen Sammeltouren haben mich Christiane Ritz, Volker Wissemann und Konstanze Bandmann begleitet. Einen ganzen Berg von Material, Daten und Ideen hat mir Katrin Klinge überlassen. Ein Großteil meiner Rosengallen wurde mir von Freunden, Entomologen, Botanischen Gärten, Experten und Wissenschaftlern in Deutschland und dem europäischen Ausland mitgebracht oder zugeschickt. Bei ihnen allen möchte ich mich herzlich bedanken und ganz besonders das außergewöhnliche Engagement von Olivier Plantard, Jojanneke Bijkerk, Maggie Frankum and Henning Adsersen hervorheben.

Die stupideste Arbeit, das Sortieren meines Probenmaterials, haben mir Sandra Schneider und Antje Schmidt abgenommen. Ohne Laborprobleme wäre eine Doktorarbeit keine Herausforderung, dem Rätsel der AFLPs konnte ich trotz der kompetenten Hilfe von Andrea Vaupel, Kathrin Ackermann, Julia Franke und Alexandra Kellner nicht endgültig auf die Schliche kommen. Wie am Schnürchen dagegen liefen die Sequenzierungen, die vor allem Iris Richter in ihrer Bachelorarbeit vorangetrieben hat.

Hilfreiche Kommentare und Korrekturen der einzelnen Teile meiner Arbeit kamen von Karen Brune, Martin Brändle, Andrea Vaupel, Roman Fricke, Jork Meyer, Kathrin Ackermann, Pete Bowen, Christian Hof und Mischa Schmidt.

Für lustige Kaffeepausen, Kulturabende, Ablenkung und Aufmunterung danke ich allen (heutigen und ehemaligen) Mitgliedern (inklusive tierischen Gästen) unserer Multifunktions-AG. Vor allem meine Büromitbewohner, allen voran Urs Gießelmann, mussten mich täglich

ertragen und mein Gejammer und Gemecker über sich ergehen lassen. Aber sonnengetrocknete Motivation von Kirsten Bogatz und Schokolade oder Kuchen helfen immer.

Die Finanzierung meiner Arbeit wurde zwei Jahre von der Deutschen Forschungsgemeinschaft (DFG) im Schwerpunktprogramm „Adaptive Radiationen – Genese biologischer Vielfalt“ (SPP 1127) getragen und ein drittes Jahr wurde mein Lebensunterhalt von der FAZIT-Stiftung Gemeinnützige Verlagsgesellschaft mbH bestritten.

Als letztes möchte ich den zahlreichen Bewohnern unserer Aquarien danken, denn durch sie habe ich in den letzten 3 ½ Jahren gelernt:

*„Ein Leben ohne Axolotl ist möglich, aber sinnlos!“*

verändert nach Loriot

# Lebenslauf

## Persönliches

| | |
|---|---|
| Name | Annette Maria Kohnen |
| Geburtstag | 01.12.1980 |
| Geburtsort | Darmstadt |
| Familienstand | ledig, keine Kinder |

## Ausbildung

| | |
|---|---|
| 1991-2000 | Besuch des Georg-Büchner-Gymnasiums in Darmstadt |
| 2000-2006 | Studium der Biologie an der Philipps-Universität, Marburg Schwerpunktfächer: Tierökologie, Zoologie, Paläontologie, Genetik |
| 2005-2006 | Diplomarbeit in der AG *Allgemeine Ökologie und Tierökologie* an der Philipps-Universität Marburg |
| ab 2006 | Promotionsstudium am Fachbereich Tierökologie der Philipps-Universität Marburg |

## Beruflicher Werdegang

| | |
|---|---|
| 2006-2007 | Wissenschaftliche Mitarbeiterin der Philipps-Universität Marburg im DFG-Projekt „Radiation, biodiversity and host-parasite interaction in the rosa-system“ (SPP 1127) |
| 2007-2008 | Wissenschaftliche Mitarbeiterin der Friedrich-Schiller-Universität Jena im DFG-Projekt „Radiation, biodiversity and host-parasite interaction in the rosa-system“ (SPP 1127) |
| 2008-2009 | Stipendium der FAZIT-Stiftung |
| ab 2009 | Wissenschaftliche Mitarbeiterin der Phillipps-Universität Marburg im BIOTA-Süd Projekt |

Marburg, Oktober 2009

# Publikationsliste

## Publikationen

Meyer, J., Kohnen, A., Harf, R., Froeschke, G., Brandl, R., (2006): Molecular markers for some small mammals of southern Africa. *Folia Zool.*, **55** (4), 444-447.

Kohnen, A., Wissemann, V., Brandl, R., (2009): No genetic differentiation in rose infesting fruit flies (*Rhagoletis alternata* Fall. and *Carpomya schineri* Loew) across Central Europe. *European Journal of Entomology*, **106** (2), 315-321

Meyer, J., Kohnen, A., Durka, W.,Wöstemeyer, J., Blaum, N., Rossmanith, E., Brandl, R., (2009): Gene flow and spatial genetic structure in the hairy-footed gerbil *Gerbillurus paeba* as revealed by microsatellite markers. *Mammalian Biology*, **74** (6), 478-487

Meyer, J., Kohnen, A., Brandl, R., (in press): Genetic structure, gene flow and population expansion in an arboreal savannah rodent. *Journal of African Zoology*

Kohnen, A., Wissemann, V., Brandl, R., (submitted): No host-associated differentiation in the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae) on three dog rose species. *Biological Journal of the Linnean Society*

## Buchbeitrag

Kohnen, A. et al. (in press): *Radiation, biological diversity and host-parasite interactions in wildroses, rust fungi and insects.* In: Glaubrecht, M. & Schneider, H. (eds): Evolution in Action - Adaptive Radiations and the Origins of Biodiversity. Springer Verlag. Berlin

## Poster

Kohnen, A., Meyer, J., Brandl, R. (2006): Phylogeography and time of divergence in the genus *Thallomys* (Rodentia, Muridae). *Mammalian Biology*, **71**, 16

Kohnen, A., Meyer, J., Brandl, R. (2006): Population structure and gene flow in the accacia rat *Thallomys nigricauda* (Rodentia, Muridae). *Mammalian Biology*, **71**, 16

Vaupel, A., Kohnen, A., Brandl, R. (2007): Low genetic differentiation between populations of the European rose hip fly *Rhagoletis alternata*. *Verhandlungen der Gesellschaft für Ökologie*, **37**, 162

Kohnen, A., Klinge, K., Wissemann, V., Brandl, R. (2008): Cynipid-parasitoid interactions: Comparing three dog rose species along a geographical gradient. *Verhandlungen der Gesellschaft für Ökologie*, **38**, 488

## Vorträge

Kohnen, A., Meyer, J., Brandl, R. (2006): Phylogeography and time of divergence in the genus *Thallomys* (Rodentia, Muridae). *Mammalian Biology*, **71**, 16

Kohnen, A., Klinge, K., Wissemann, V., Brandl, R. (2008): Cynipid-parasitoid interactions: Comparing three dog rose species along a geographical gradient. *ICE 2008 Programm & Exhibitor directory*, 178